THE SIGNALING FUNCTION OF PLUMAGE CHARACTERISTICS IN FEMALE TREE SWALLOWS AND IMPLICATIONS FOR REPRODUCTIVE SUCCESS AND LIFE-HISTORY DECISIONS

by

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B.Sc., Carleton University, 2006

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
NATURAL RESOURCES AND ENVIRONMENTAL STUDIES
(BIOLOGY)

UNIVERSITY OF NORTHERN BRITISH COLUMBIA

April 2011

Your file Votre référence
ISBN: 978-0-494-75157-2
Our file Notre référence
ISBN: 978-0-494-75157-2

Abstract

The existence of elaborate plumages in birds has traditionally been explained as a result of mate choice by females for these traits in males. Ornate plumage traits are thought to be costly to produce and maintain, allowing females to gain accurate information about potential mates based on the expression of these traits. Female plumage traits, on the other hand, are less understood and have been rarely studied as having signaling potential. In an attempt to reduce this gap in knowledge, I investigated the potential signaling function of specific plumage characteristics in female tree swallows (*Tachycineta bicolor*). Tree swallows are one of only two species of North American passerines where females, rather than males, have delayed plumage maturation (DPM). Females in their second year of life (SY) display mainly brown "subadult-coloured" feathers on their dorsal surface, while older (after-second year of life: ASY) females primarily have iridescent blue-green "adult-coloured" feathers. There is, however, much variation in the number of adult-coloured feathers in the dorsal plumage of SY females. To date, only one study has experimentally examined the potential signaling function of DPM in female tree swallows, and no study has yet examined variation in the amount of DPM exhibited among SY females. Although plumage colour of ASY female tree swallows is less variable compared to SY females, such variation in ASY females is related to reproductive success, making both SY and ASY female plumage characteristics potentially indicative of quality. In this thesis, I investigated 1) if conspecifics behave differently towards SY and ASY females based solely on plumage colouration, indicating that DPM could signal subordinate status and inexperience during the breeding season, 2) if variability in the amount of DPM in SY female tree swallows is a condition-dependent trait and therefore possibly a target of sexual selection, and 3) whether variation in plumage traits of

ASY female tree swallows is related to quality and thus could indicate how females choose to balance investment between life-history traits in this species. I found that the subadult-coloured plumage of SY female tree swallows elicits significantly less aggression from resident female tree swallows during the nest-building stage of reproduction compared to the adult-coloured plumage of ASY females. These results suggest that the plumage of SY females is an adaptive trait that signals subordinate status to conspecific females, potentially reducing intrasexual competition for SY females during the breeding season, increasing their chances of successfully breeding. Separately, when examining variation in the amount of DPM exhibited by SY females, I found that SY females with more adult-coloured plumage were in poorer body condition, had lower reproductive success and were mated to less UV-blue coloured males. I propose that increased agonistic encounters may account for the reduced reproductive success and condition of more adult-coloured SY females in my study. When examining the condition-dependence of ASY female plumage characteristics, I found that ASY females with more UV-blue colouration had lower reproductive success, while those with more UV-blue and brighter plumage had more parasites in their nests. These results, although unexpected, may still agree with current knowledge of the signaling function of ornamentation in birds if ASY females are experiencing costs associated with higher social status or sexual selection. Overall, my findings demonstrate that the adult-coloured plumage of female tree swallows is a potentially costly trait that elicits increased intrasexual aggression. As SY females with less adult-coloured plumage and ASY females with less ornamented adult-coloured plumage had higher reproductive success, my findings demonstrate an adaptive function of less ornamentation that has rarely been reported, increasing the current knowledge on the communicative function of plumage ornamentation.

Acknowledgements

This Master's thesis would not have been possible without the constant encouragement and support from my supervisor, Dr. Russell D. Dawson. Russ has always provided me with new avenues to explore in my research, given me confidence in my abilities and has been very supportive throughout my entire time here at UNBC. I feel that I have grown immensely from this experience, both personally and professionally, due in large to his style of independent supervising, and for that I am grateful. I am very thankful for my committee members Drs. Mark Shrimpton and Stephen Rader for valuable input on this project and for the use of their laboratories and equipment. I would also like to thank Dr. Colleen Barber for being my external examiner and providing valuable feedback on my thesis.

I am grateful to all of my lab colleagues for all of the guidance and help they have provided during my time at UNBC. Erin O'Brien, Jennifer Greenwood and Lisha Berzins have all greatly helped me with my research, from help with statistical analyses to our discussions during our "lab meetings", scheduled and impromptu, they have all been tremendous pillars of support. I will always cherish our time together in the lab.

I am also thankful to all of the people that have assisted me in the field during my two field seasons, Nicole Johnstone, Leah Hurrell, Cory Ochs, Jessica Milko, Robijn VanderZwan and Sarina Loots. I also thank D. and K. Steward, Y. Wallace and G. Sanders, for giving me access to their properties to conduct these studies.

I also thank my family for always believing in me and encouraging me to achieve my goals. Without their support, both financially an emotionally, I would not have completed this Master's thesis.

Funding support for this project was provided by the Natural Sciences and Engineering Research Council of Canada, Canada Foundation for Innovation, British Columbia Knowledge Development Fund, and the University of Northern British Columbia through grants to R.D. Dawson and a Research Project Award provided to me through UNBC. All research protocols were approved by the Animal Care and Use Committee of UNBC.

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1 General Introduction

Sexual selection theory proposes that ornamental traits have primarily evolved to communicate individual quality to potential mates (Darwin 1871; Andersson 1994). In addition to communicating to potential mates, in species that compete for limited resources or mating opportunities, ornamental traits may also have intrasexual social functions, signaling the bearer's threat as a competitor or their social status within a dominance hierarchy (reviewed in Senar 2006). Ornamental traits are considered to be "honest" advertisements of individual quality because of the differential costs, such as energetic or social costs (Hamilton and Zuk 1982; Kodric-Brown and Brown 1984), associated with their acquisition and maintenance. The elaborately coloured plumage of males in many species of birds has been the focus of numerous investigations on the type of information being conveyed by ornamental traits. Plumage colouration is highly variable among species, from the vermilion red plumage colouration of male northern cardinals *(Cardinalis cardinalis)* (Halkin and Linville 1999) to the iridescent ultra violet-blue throat colouration of male bluethroats (*Luscinia s. svecica*) (Andersson and Amundson 1997). It is the variation in plumage colouration *within* species, however, that has sparked researchers' interest in the type of information being conveyed.

Although the conspicuous plumage colouration of male birds initially provoked the interest of researchers, evidence is accruing that female plumage colouration may have both inter- (Roulin et al. 2001; Siefferman and Hill 2005) and intrasexual (Johnsen et al. 1996) signaling functions. Studies, however, are still lacking that examine the signaling potential of female plumage colouration, potentially due to the assumption that female ornaments are

merely genetic correlates of male ornaments (Lande 1980), or that there is little competition between females to attract mates, as conventional ideas about sex roles regard the female as the choosier sex (Andersson 1994).

There are two main mechanisms of plumage colouration; pigment-based colouration, often produced by melanin and carotenoids pigments, and structural-based colouration, produced from the interaction of light with the feather nanostructure (Osorio and Ham 2002; Prum 2006). Pigment-based colouration is commonly studied as having signaling potential due to the physiological mechanisms linking pigment-based colouration to individual condition (McGraw et al. 2001; Tschirrin et al. 2003; McGraw 2007; Bokony et al. 2008). The physiological mechanisms regulating the variation in structural plumage colouration, however, are not as well understood (Keyser and Hill 1999; Prum 2006). Only recently has structural plumage colouration received attention as a condition-dependent signal. Studies have found that variation in structurally-coloured plumage is related to the nutritional status (Keyser and Hill 1999) and physiological condition of the bearer (Doucet and Montgomerie 2003), potentially due to the costly nature of producing fine nanoscale feather structures (Shawkey et al. 2003; Doucet et al. 2006). Bitton et al. (2008) examined the potential signaling function of structural plumage colouration in female tree swallows (*Tachycineta bicolor*), and found that ultraviolet (UV) -blue colouration and plumage brightness were related to reproductive success and were potentially sexually selected traits. To date, theirs is the only study that has examined plumage characteristics in female tree swallows, and their analyses were limited to females that were older than their second year of life (after-second year: ASY). A need therefore exists for more studies examining the signaling function and

potential condition-dependence of female plumage characteristics in this species, especially among younger individuals.

Birds usually attain adult-type plumage at the end of their post-juvenile moult, however, in more than 30 species of North American passerines, males will moult into a less-elaborate, "subadult" plumage, a phenomenon known as delayed plumage maturation (DPM) (Rohwer et al. 1980). DPM is thought to have mainly evolved under circumstances where male competition for resources or mates is high, allowing younger males to honestly signal their inexperience and lowered competitive threat to older males, thus avoiding conspecific aggression (reviewed in Senar 2006). Tree swallows, however, are one of only two species of North American passerines where young females in their second year (SY) of life, rather than males, exhibit DPM (Morton 1989). Of the few studies that have examined a communicative function of DPM in female tree swallows, it has been suggested that DPM reduces aggression during the breeding season by signaling female-status to males (Stutchbury and Robertson 1987a) or age and dominance status to ASY females (Lozano and Handford 1995). There is, however, much variation in the amount of DPM exhibited within SY females (details below), suggesting that DPM may also signal some aspects of individual quality (Møller and Hoglund 1991; Rowe and Houle 1996).

The goal of my research was to determine the potential signaling function of female plumage traits in tree swallows and whether variation in plumage characteristics within age classes is related to individual quality. To do this, I examined 1) if the subadult plumage of SY female tree swallows signaled subordinate status during the breeding season and adaptively reduced conspecific aggression, or if DPM is disadvantageous by signaling inexperience in territory defense, 2) if the variability in the amount of DPM was a condition-

dependent trait in SY female tree swallows and therefore subject to sexual selection and 3) whether variation in plumage characteristics of older (i.e. ASY) female tree swallows was related to individual quality and thus could be indicative of how an individual choses to balance investment between self-maintenance and reproduction.

1.1 Study species and study area

Tree swallows are small (ca. 21 g), monomorphic, migratory passerines that breed throughout most of northern and central North America (Robertson et al. 1992). Tree swallows are obligate secondary cavity nesters and compete intensely for breeding opportunities. Many reproductively mature individuals do not breed and are instead "floaters" within the population. Floaters may exist in a population for several reasons (Penteriani et al. 2011). One such reason is that they are competitively excluded by conspecifics from breeding (Stutchbury and Robertson 1985; Penteriani et al. 2011). Although males arrive on the breeding grounds first in spring and compete for nest sites, female tree swallows are highly aggressive and territorial during the breeding season, with intrasexual encounters often leading to injury, or in some cases death, in defense of suitable nest sites and mates (Leffelaar and Robertson 1985; CD Coady personal observation).

Shortly after the breeding season, from early August until late September, tree swallows undergo a pre-basic moult and develop their new plumage before migrating south to the wintering grounds (Robertson et al. 1992). Males hatching in the current year (hatch year) undergo a post-juvenile moult that results in iridescent blue-green (adult-coloured) plumage, making them indistinguishable from older males. Hatch-year females, however, exhibit DPM and their post-juvenile moult results in them displaying a brown "subadult"

plumage during their second year of life. There is also much variation in the amount of DPM that is exhibited by SY female tree swallows, and Hussell (1983) reported that SY females varied from completely brown to 50% iridescent blue-green upperparts, with rare instances of SY females displaying fully iridescent blue-green plumage. It is not until their next moult, a year later, when females obtain the complete iridescent blue-green plumage similar to males (Robertson et al. 1992).

My four study sites for this research were located within a 25 km radius of Prince George, British Columbia. Study sites consisted of open agricultural fields mixed with patches of coniferous and deciduous forest, and small wetlands. My two main study sites have had established tree swallow colonies since 2001, where approximately 60 - 75 pairs of tree swallows breed in nest boxes each year. Two additional sites were established in 2008 and approximately 20 - 40 pairs of tree swallows breed in nest boxes each year.

1.2 General objectives

1.2.1 Potential signaling function of DPM in SY female tree swallows

Most commonly exhibited by males, delayed plumage maturation (DPM) is defined as the delay in the acquisition of adult traits without a delay in sexual maturity (Rohwer et al. 1980). Breeding and non-breeding season hypotheses for the adaptive significance of DPM suggest that it may have evolved by communicating a lowered competitive threat to conspecifics (reviewed in Beauchamp 2003). As a result, DPM may thus reduce conspecific aggression and competition for mates and resources, increasing the fitness of the bearer (Lyon and Montgomerie 1986; Rohwer and Butcher 1988).

Of the few studies that have investigated the functional significance of DPM in SY female tree swallows (Stutchbury and Robertson 1987a; Lozano and Handford 1995), Stutchbury and Robertson (1987a) suggested that DPM may adaptively signal female gender to males. They found that males were less aggressive towards intruding SY females compared to ASY females, potentially indicating that subadult plumage may aid in nest-site exploration for these females, many of which do not breed in their first potential breeding season due to competition from more experienced ASY females (Stutchbury and Robertson 1985). Alternatively, while displaying subadult plumage could be beneficial for SY females, Whittingham and Schwabl (2002) found that SY female tree swallows experienced more nest-site intrusions compared to ASY females. Therefore, SY females may be displaying their inexperience in territory and nest defense via their plumage colouration, creating the perception of an easily attainable nest site to intruders.

In Chapter 2, I test whether the subadult plumage of SY female tree swallows is adaptive during interactions with conspecifics by advertising subordinate status and inexperience, thus reducing aggression for SY females during the breeding season. As tree swallows actively search for nest sites and mates, and previous research suggests that DPM may aid in nest-site exploration (Stutchbury and Robertson 1987a), I wanted to determine if nest-site owners behave differently towards intruding female tree swallows based on plumage colouration. Alternatively, displaying subadult plumage may be disadvantageous to SY female tree swallows that occupy nest sites. Their plumage may display their inexperience in territory and nest defense, creating the perception of an easily attainable nest site to intruders and lowering their potential for successful breeding events. Thus, I also wanted to investigate if tree swallows behave differently towards female territory owners

based on differences in female plumage colouration. These experiments thus enabled me to determine how conspecifics perceive females with subadult plumage and determine any potential adaptive function of DPM during the breeding season. My work will greatly contribute to the limited understanding for the existence of DPM in this species, and may also contribute new findings to current understanding for the evolutionary significance of DPM in other species.

1.2.2 Variation in plumage as an indicator of individual quality

Condition-dependent traits are considered to be indicators of individual quality because differential costs, such as energetic or social costs (Kodric-Brown and Brown 1984; Andersson 1994), are associated with the acquisition and maintenance of these traits to ensure the signaler is communicating an "honest" signal to the potential receiver(s). Variation in plumage colouration is thought to have evolved as a sexually selected trait (Darwin 1871; Andersson 1994), honestly conveying aspects of individual quality such as the bearer's physical condition, reproductive capabilities or genetic characteristics (e.g., Norris 1993; Siefferman and Hill 2003). DPM, however, is a plumage characteristic that is not considered to have evolved by sexual selection, rather it is thought to communicate low competitive threat to conspecifics by honestly signaling the bearer's age and breeding inexperience (reviewed in Senar 2006). Of the species that exhibit DPM, however, those that most resemble older individuals commonly have higher mating and breeding success compared to individuals with less adult-coloured plumage (Hill 1988; Grant 1990). This suggests that in addition to being a signal of age or social status in intrasexual interactions, DPM may also serve as an intersexual signal of individual quality.

SY female tree swallows can display variable numbers of iridescent blue-green feathers on their dorsal surface, and Hussell (1983) reported that SY females varied from completely brown to 50% iridescent blue-green upperparts, with rare instances of SY females displaying fully iridescent blue-green plumage. As signals of quality are known to have higher degrees of variability than non-signaling morphological traits (Møller and Hoglund 1991; Rowe and Houle 1996), the marked variation in plumage colouration among SY female tree swallows could be attributed to differences in individual quality, yet the reflective properties of their plumage have not been reported.

In Chapter 3, I examine relationships between SY female plumage colouration and measures of individual quality; specifically examining female body condition, reproductive investment and reproductive success. I also investigate male reproductive investment in relation to SY female plumage colouration and whether pairs mate assortatively by plumage characteristics, which may indicate that males are assessing female quality based on plumage colouration. To my knowledge, DPM has never been examined as an indicator of quality and thus the variation in DPM among individuals has rarely been examined (but see Hill 1988; Grant 1990; Greene et al. 2000). By examining the variation within this plumage trait, I am seeking to understand if there are selective forces not normally considered that may have influenced the existence of DPM in this species. This work therefore takes a new approach to understanding the existence of DPM and may lead to future work examining DPM in other species from this uncommon perspective.

1.2.3 Variation in female plumage colouration as an indicator of life-history trade-offs

Trade-offs are a central concept in life-history theory and one such trade-off is an organism's decision to allocate resources to current reproduction versus survival and future reproduction (Williams 1966; Stearns 1992). The underlying mechanisms mediating these trade-offs appear to be highly variable among species (Gustaffson et al. 1994; Ardia and Clotfelter 2005; Monaghan et al. 2009). In tree swallows, previous research has found that individual quality, as measured by clutch initiation date, was a mediating factor in the decision for females to invest in reproduction or in immune function, with higher quality females showing less of a trade-off between these two costly physiological processes (Ardia 2005a).

In many bird species, plumage colouration is considered a condition-dependent signal of quality because the costs associated with the production and maintenance of more elaborate plumage colouration (Kodric-Brown and Brown 1984; Andersson 1994). ASY female tree swallows have iridescent blue-green feathers on their dorsal surface, which are coloured via the interaction of light with the feather nanostructure (Osorio and Ham 2002). Although not as apparent as the variation in plumage colouration within SY female tree swallows (Hussell 1983), previous research has found that UV-blue colouration increased with age in ASY female tree swallows and that pairs mated assortatively for plumage brightness, suggesting that structural plumage colouration may be related to female quality and may be a sexually selected trait in this species (Bitton et al. 2008).

Although the physiological mechanisms regulating the variation in structural plumage colouration are not well understood (Keyser and Hill 1999; Prum 2006), studies have found that variation in the hue and brightness of structurally-coloured feathers is related to the nutritional status (Keyser and Hill 1999) and physiological condition of the bearer (Doucet

and Montgomerie 2003). The costly nature of producing these fine nanoscale feather structures may be one potential mechanism driving this condition dependence (Shawkey et al. 2003; Doucet et al. 2006). If plumage colouration is related to female quality in tree swallows, it could indicate how females will balance trade-offs between investment in self-maintenance and reproduction.

In Chapter 4, I investigate whether variation in structural plumage colouration of ASY females is indicative of individual quality. As trade-offs may be more evident under stressful conditions (Friedl and Edler 2005; O'Brien and Dawson 2008), I also examine if plumage colouration is indicative of how ASY females choose to balance investment in self-maintenance versus reproduction when under the physiological stress of an immune challenge. Life-history trade-offs are important processes that shape the evolution of species. Characteristics that may indicate individual quality, such as plumage colouration, may shed light on how individuals choose to balance such trade-offs, and thus further our understanding of evolutionary processes in birds.

2. Subadult plumage of female tree swallows reduces conspecific aggression during the breeding season

2.1 Abstract

Delayed plumage maturation (DPM) in birds is a delay in the acquisition of adult-type plumage without a delay in sexual maturity and has been found to be adaptive for males breeding for the first time by reducing aggression from older conspecifics. Tree swallows (*Tachycineta bicolor*) are one of only two species of North American passerines where females in their second year of life (SY) rather than males display DPM. The functional significance for DPM in females remains equivocal. I examined potential advantages and disadvantages of displaying DPM during the breeding season by testing whether male and female tree swallows behave differently towards SY and ASY (after second year of life: ASY) female tree swallow models when presented both as territory 'intruders' and as territory 'owners'. I predicted that resident pairs would behave more aggressively towards the ASY (adult-coloured) model when presented as an intruder if ASY females are perceived as a greater competitive threat. I further predicted that the SY (subadult-coloured) model would have a greater rate of intrusions when presented as a territory owner if conspecifics perceive subadult plumage as a signal of inexperience in territory defense. When models were presented as intruders in occupied territories I found that resident females were more aggressive towards those with adult-coloured plumage compared to those with subadult-coloured plumage, while behaviour of resident males did not differ according to the age of the models. When models were presented as territory owners, I found that the ASY model was subjected to significantly more aggression from intruding tree swallows, although there was no effect of model age on the number of intrusions into the model territories or the

number of nest box inspections. I suspect that intruders were neighboring tree swallows that were defending their own territories as opposed to floaters attempting to usurp the territory "occupied" by the models. My results suggest that subadult plumage in this species is an adaptive trait that signals subordinate status to conspecific females, potentially reducing intrasexual competition for SY females during the breeding season.

2.2 Introduction

Much attention has focused on the fitness benefits of elaborate plumage of male birds with respect to sexual signaling (reviewed in Senar 2006). Plumage ornaments, however, have also been found to signal such characteristics as age, aggressiveness or social-dominance (Korsten et al. 2007; Quesada and Senar 2007). Plumage, therefore, may potentially display individual quality and degree of threat as a competitor to conspecifics. Delayed plumage maturation (DPM) is most commonly exhibited by males and is defined as the delay in the acquisition of adult plumage traits without a delay in sexual maturity, resulting in a 'subadult' plumage (Rohwer et al. 1980). Breeding and non-breeding season hypotheses for the adaptive significance of DPM suggest that it may have evolved by communicating a lowered competitive threat to conspecifics and thus reducing conspecific aggression and competition for mates and resources, increasing the fitness of the bearer (Lyon and Montgomerie 1986; Rohwer and Butcher 1988).

The status-signaling hypothesis is arguably the most well supported of the breeding season hypotheses and suggests that DPM exhibited by males in their second year of life (SY) honestly signals their subordinate status to older, after second year of life (ASY) males. DPM may thus result in a reduction in male aggression towards SYs, potentially allowing SY

males easier access to females (Lyon and Montgomerie 1986). Pertaining specifically to dichromatic species, the female mimicry hypothesis suggests that DPM may deceptively communicate female-status to ASY males thus allowing SY males to avoid costly agonistic interactions with ASY males (Rohwer et al. 1980). Regardless of the mechanism, both of these hypotheses suggest that DPM communicates a lowered competitive threat to conspecifics. While there is evidence to support the potential adaptive functions of DPM (reviewed in Beauchamp 2003), several studies have shown that individuals displaying DPM have reduced reproductive success and obtain fewer resources (Enstrom 1992; Conover et al. 2000), illustrating the necessity of investigating both potential advantages and disadvantages of DPM in a given study.

Tree swallows (*Tachycineta bicolor*) are one of only two species of North American passerines where females exhibit DPM rather than males (Morton 1989). SY females have subadult dorsal plumage that is predominately brown, although up to 50% of these feathers may be iridescent blue-green, while ASY females, as well as all males, have predominately iridescent blue-green dorsal plumage (Hussell 1983). Of the few studies that have investigated the functional significance of DPM in female tree swallows (Stutchbury and Robertson 1987a; Lozano and Handford 1995), Stutchbury and Robertson (1987a) suggested a gender-signaling hypothesis for the adaptive significance of subadult plumage in tree swallows. Stutchbury and Robertson (1987a) found that males were less aggressive towards intruding SY females compared to ASY females. These results suggest that subadult plumage may aid in nest-site exploration for these females, many of which do not breed in their first potential breeding season due to competition from more experienced ASY females (Stutchbury and Robertson 1985).

Tree swallows are obligate cavity nesters that frequently use artificial nest boxes. Although fierce competition for nest sites among males begins early in the breeding season (Kuerzi 1941), with males returning to the breeding grounds prior to females and claiming a nest site (Cohen 1987), females also participate in aggressive interactions as they compete for suitable mates and nest sites. Since many nest sites can remain unoccupied throughout the breeding season, it is believed that females compete for males with nest sites rather than vacant nest sites alone (Leffelaar and Robertson 1985), and aggressive interactions among females can lead to severe injury or death (Leffelaar and Robertson 1985; CD Coady personal observation). As DPM has been found to function by reducing intrasexual aggression during the breeding season in males of many species (reviewed in Lyon and Montgomerie 1986), DPM in female tree swallows could also function by reducing aggression from conspecific females. Although Stutchbury and Robertson (1987a) found that resident female tree swallows were equally aggressive to female intruders regardless of age, other studies have commonly found female ornaments to be signals of status in species with high levels of female-female competition for resources and mates (Johnsen et al. 1996; Murphy et al. 2009). As Stutchbury and Robertson's (1987a) study is the only study to experimentally examine the potential signaling function of DPM in tree swallows, the hypothesis that DPM is an adaptive intrasexual signal of subordinance in SY female tree swallows is worthy of further investigation.

While displaying subadult plumage could be beneficial for young females, Whittingham and Schwabl (2002) found that SY female tree swallows experienced more nest-site intrusions compared to ASY females. SY females therefore may be displaying their inexperience in territory and nest defense via their plumage colouration, creating the

perception of an easily attainable nest site to intruders. This notion stems from the theory of contest asymmetries, where individuals with more elaborate ornaments should be less contested by conspecifics because their perceived condition is greater compared to less-ornamented individuals (Reichert 1998). In song sparrows (*Melospiza melodia*) for example, younger, more inexperienced males are poorer territory defenders (Hyman et al. 2004) and are more frequently intruded upon by unmated 'floating' males (Arcese 1987). If plumage colouration is an intrasexual signal of competitive fighting ability as seen in other species (Hegyi et al. 2008b), SY female tree swallows may be perceived as poor territory defenders by intruders, whereas potential intruders may decide to avoid territories occupied by ASY females if the intruder has a greater risk of losing a contest (Rohwer 1982). As there has been relatively little research carried out on the functional significance of DPM in tree swallows (Stutchbury and Robertson 1987a; Lozano and Handford 1995), I investigated whether resident tree swallows perceive SY and ASY females differently during the breeding season. Stutchbury and Robertson (1987a) also studied a relatively dense population of tree swallows with a high frequency of intrusions into nest sites, and a known 'floating' population of females that were unable to acquire nest sites (Stutchbury and Robertson, 1985). In my study area, competition for nest sites is potentially less pronounced, with as many as 45% of the nest sites remaining vacant for the duration of the breeding season (O'Brien and Dawson 2007), potentially allowing for the benefits of subadult plumage during intrasexual interactions to be more evident. I also examined whether subadult plumage of SY female tree swallows was perceived by competing tree swallows as a signal of inexperience in nest-site defense (Whittingham and Schwabl 2002), which may indicate that DPM in tree swallows

had signaling potential during other periods of the annual cycle (reviewed in Beauchamp 2003).

2.3 Methods

I studied tree swallows breeding in nest boxes during 2008 at two study sites; one located 25 km west of Prince George, BC, Canada (53°50'N, 123°2'W), and the other 25 km south of Prince George (53°45'N, 122°33'W). Both study sites consisted of open agricultural fields mixed with patches of coniferous and deciduous forest, and small wetlands. Experiments were performed between 09:00 and 16:00 from early to mid- May, when tree swallows in my region were establishing territories and building nests.

2.3.1 Model intruder plumage colour and resident behaviour

To investigate whether the subadult plumage of SY females reduces aggression from conspecifics during the breeding season, I placed both SY and ASY female models within resident pair territories during two experiments (details below). I hypothesized that the ASY model would receive more aggressive and defensive behaviours from established pairs of tree swallows in comparison to the SY model, which could indicate that adult-coloured plumage is a signal of higher competitive threat to conspecifics during the breeding season. The amount of aggression elicited from a resident pair was measured based on the number of times a male or female resident attacked or hovered over each model. I was unable to time the length of each hover or attack, however, a hover was considered so if a tree swallow was directly above the model for at least two seconds and was no further than 60 cm above the

model. An attack was considered to have occurred if a tree swallow visibly contacted the model.

The first experiment investigated the responses of resident pairs to the SY and ASY models when presented simultaneously. Presentations were 5 minutes in length and models were placed on 1.5 m high wooden poles approximately 1.5 m apart and 1.5 m in front of resident pairs' nest boxes, facing the nest box to simulate a territorial intrusion, similar to the design of Stutchbury and Robertson (1987a). The position of the models to the left and right was alternated between observations, and models were only presented once to any given resident pair. The second experiment examined the response of resident pairs when models were presented separately. Presentations were 5 minutes in length and models were mounted directly on top of nest boxes to simulate more aggressive intrusions into territories. The order that the models were presented was alternated between presentations, and the time between SY and ASY model presentations for a given resident pair ranged from 1.5 to 3.5 hours.

2.3.2 Model resident plumage colour and intruder behaviour

To investigate if intruders respond differently according to the age of resident female tree swallows, which could indicate differences in intruder perception of the nest defense capabilities of female tree swallows, I separately placed both SY and ASY female models in unoccupied territories for 20 minutes to simulate their ownership of that territory. Some presentations, however, had to be abruptly stopped if intruders began to severely attack the models, although in no cases were models visibly damaged before a trial was stopped. Models were placed on a nest box attached to a metal pole or a fence post approximately 1.5 m high and approximately 20 m from already established pairs. I attempted to minimize the

influence of neighboring tree swallows entering my simulated territory in defense of their own by remaining outside the maximum range that tree swallows have been found to defend (Robertson and Gibbs 1982), while ensuring that my model territory was still visible to tree swallows in my study area. A neighboring nest box was considered 'established' if a pair was seen occupying the area in the vicinity of the nest box (i.e. within ~10 m around the nest box), and if the nest was approximately midway or further though the nest-building stage. Each time a tree swallow entered the model territory (i.e. within ~10 m of the model mounted to the nest box), I used a 6 point scoring system (Table 2.1) similar to Duckworth (2006) and assigned a single score to each individual to categorize the nature of the intrusion, with higher scores representing the most aggressive intrusions. As multiple tree swallows would often simultaneously enter the territory of the models, it was not always possible to track an individual intruder if it left the territory; therefore, if an intruder left and later returned to the territory, it was treated as a separate intrusion. As some presentations did not last a full 20 minutes due to the model being attacked (see above), I calculated a mean intrusion score for each model presentation at the end of the observation period. Similarly, for each presentation, I also calculated the number of scores that included attacks on the model (i.e. score of 4 or higher; see Table 2.1) and divided that by the total number of scores for a given presentation to get the proportion of scores that included attacks on the models. I was often unable to identify the sex of the individual intruders due to multiple tree swallows simultaneously entering the model territory, however, only during four of the presentations did I identify an SY female entering the model territory, therefore the remaining intruders were either males or ASY females.

Table 2.1 Intruder behaviour scoring system for model-occupied territories of tree swallows (adapted from Duckworth 2006).

Score	'Intruder' behaviour and distance from nest box	Number of 'intruder' attacks on model
1	10-5 metres from nest box	0
2	<5m from nest box/hovering over model	0
3	Inspecting interior of nest box	0
4	Sitting on nest box and/or hovering over	1 to 2
5	Sitting on nest box and/or hovering over	3 to 5
6	Sitting on nest box and/or hovering over	>5

For experiments on both resident and intruder behaviour, observations were made from a distance of ~30 m from the model set up and as far as possible from any neighboring nest sites. Observations were dictated into digital recorders and later transcribed.

2.4 Statistical analyses

2.4.1 Intrusion experiments

To investigate whether resident birds responded differently to SY and ASY female models, I separately analysed the number of hovers and attacks on models using a repeated measures design, with SY and ASY models as the repeated measure. In separate analyses I examined the behaviour of resident males, resident females, and both sexes combined. As residents were not captured and marked prior to presentations, the sex of residents could not always be identified, and so sample sizes for analyses of the individual male and female residents are smaller compared to when the pair was examined together.

Tree swallows are known to be most active and defensive around their nest sites in the early morning when the majority of territorial behaviour, courtship and nest building occurs (Cohen 1984a; Whittingham et al. 1994). Consequently I used the start time of the presentation and the stage of the nest construction as covariates in the analyses. Stage of nest construction was scored on a 5 point scale, ranging from a few blades of grass (1) to a completely constructed nest (5). Both intrusion experiments were performed over only a 2-day period, so I did not examine how behaviour varied with presentation date. When I presented the models directly on the nest boxes of resident pairs to simulate a more aggressive territorial intrusion, I also included which model (SY or ASY) was presented first and the elapsed time between the presentations, which could influence factors such as the

hormonal levels of the resident pairs and thus the aggressiveness of their responses to the intruding models (reviewed in Wingfield et al. 1987). I classified resident females as SY or ASY by their plumage colouration (Hussell 1983), and included female age as a factor in the analyses. Observations where the pair did not respond to either model (i.e. no hovers or attacks, $n = 4$) were excluded from the analyses, as I cannot be certain that the residents were aware of the models' presence within their territories.

2.4.2 Resident experiment

I used analyses of covariance (ANCOVA) to examine the effects of model age on the mean intrusion scores, proportion of scores that included attacks per presentation, proportion of scores per presentation that included nest box inspections and number of intrusions per minute into the model territories. To meet assumptions of parametric analyses, I performed inverse-log transformations on the mean intrusion scores, and arcsine-square root transformations on the proportion of scores that included attacks on models. As I was often unable to accurately identify the sex of the intruders, I analysed data without specifically examining the behaviour of male and females individually. I examined the level of establishment of neighboring nest boxes by including the highest nest stage (on a 5 point scale; see above) of the two nest sites within closest proximity to my model territories to determine whether intrusions into my model territories could be neighboring tree swallows defending their own territories due to uncertainty of their own territory establishment. As presentations were performed over multiple days, I also included date as a covariate because more intrusions may have occurred during earlier presentation dates if more tree swallows have not yet acquired nest sites.

All statistical analyses were performed using SPSS (Norušis 2000). Results were considered significant at the 0.05 level and means are presented ± standard error (SE).

2.5 Results

2.5.1 Effects of model intruder plumage colour on resident behaviour

When the SY and ASY models were presented simultaneously on poles in front of resident pairs nest boxes, I found that resident pairs hovered over the ASY model (2.19 ± 0.42 hovers) significantly more than the SY model (1.35 ± 0.42 hovers; $F_{1, 25} = 4.42, p < 0.05$). Time of presentation, stage of nest construction and age of the resident female did not influence the results (all $p > 0.59$). When analysing the behaviour of the sexes separately, there were no significant differences in the number of hovers over the ASY versus SY model for either resident females (ASY model: 0.82 ± 0.32; SY model: 0.88 ± 0.38; $F_{1, 16} = 0.05, p = 0.83$) or males (ASY model: 1.00 ± 0.32; SY model: 0.59 ± 0.36; $F_{1, 16} = 1.91, p = 0.19$), which may be the result of reduced sample sizes as some observations were excluded when the sex of the residents could not be identified. I also found that resident pairs attacked the ASY model (3.85 ± 1.56 attacks) more than the SY model (0.39 ± 0.31 attacks; $F_{1, 19} = 11.99, p < 0.01$). SY resident females also attacked the models more compared to ASY resident females ($F_{1, 19} = 7.59, p = 0.01$). This analysis also showed that there was an interaction between model age and time of presentation ($F_{1, 19} = 7.26, p = 0.01$), and between model age and the resident pair's stage of nest construction ($F_{1, 19} = 6.41, p = 0.02$). To further investigate these interactions, I analysed data separately for each model age. I found that attacks on the ASY model decreased with time of day of presentation ($F_{1, 19} = 7.31, p = 0.01$), but no such relationship was found for the SY model ($F_{1, 19} = 0.01, p = 0.98$).

Similarly, resident pairs attacked the ASY model more often during earlier stages of nest construction ($F_{1, 19} = 5.92, p = 0.03$), while no such relationship was found for the SY model ($F_{1, 19} = 0.26, p = 0.62$).

When examining the number of resident female attacks on the models, it appears that the higher number of attacks on the ASY model from the resident pairs and the significant interactions with time of presentation and stage of nest construction were entirely due to the behaviour of the resident females. Resident females attacked the ASY model (3.32 ± 1.53 attacks) significantly more than the SY model (0.40 ± 0.24 attacks; $F_{1, 19} = 9.07, p < 0.01$), and SY resident females attacked the models more compared to ASY resident females ($F_{1, 19} = 9.21, p < 0.01$). This analysis also showed that there was an interaction between model age and time of day of presentation ($F_{1, 19} = 4.97, p = 0.04$), and between model age and the resident pair's stage of nest construction ($F_{1, 19} = 4.34, p = 0.05$). Analyses of each model age separately showed that resident female attacks on the ASY model decreased with time of day of presentation ($F_{1, 19} = 5.01, p = 0.04$), but no relationship was found for the SY model ($F_{1, 19} = 0.01, p = 0.98$). Similarly, resident females attacked the ASY model more often during earlier stages of nest construction ($F_{1, 19} = 5.24, p = 0.03$), while no relationship was found for the SY model ($F_{1, 19} = 0.26, p = 0.62$). Among the resident birds of which I was able to determine their sex, I only observed one resident male attacking the ASY model during a presentation; otherwise I did not observe resident males attacking either model during any of the presentations.

By presenting the ASY and SY models separately on resident pairs' nest boxes, I further investigated whether intruder plumage colour influenced resident behaviour by simulating more aggressive territorial intrusions. Similar to my first experiment, I found that

the resident pairs hovered significantly more over the ASY model (12.0 ± 2.59 hovers) compared to the SY model (5.23 ± 1.33 hovers; $F_{1, 10} = 8.09$, $p = 0.02$; Figure 2.1a). This relationship was influenced by which model was presented first to the pairs, such that the resident pairs hovered more over the models when the ASY model was presented first ($F_{1, 10} = 7.19$, $p = 0.02$). The age of the resident females, the time between the model presentations and the stage of nest construction did not influence the number of hovers over the models (all $p > 0.30$). Resident males alone did not differ in the number of hovers over the ASY (3.17 ± 1.45 hovers) and SY model (1.25 ± 0.69 hovers; $F_{1, 11} = 1.34$, $p = 0.27$), however, I found a trend for resident females to hover more over the ASY model (9.42 ± 2.81 hovers) compared to the SY model (4.25 ± 1.16 hovers; $F_{1, 11} = 4.22$, $p = 0.07$; Figure 2.1b). I also found that resident pairs attacked the ASY model (21.29 ± 5.28 attacks) significantly more than the SY model (2.86 ± 1.90 attacks; $F_{1, 11} = 14.22$, $p < 0.01$; Figure 2.2a) as well as a trend for resident pairs to attack the models more if the ASY model was presented first ($F_{1, 11} = 4.01$, $p = 0.07$). The behaviour of resident pairs appeared to be driven by the behaviour of resident females, who attacked the ASY model (19.93 ± 5.52 attacks) more than the SY model (2.79 ± 1.91 attacks; $F_{1, 11} = 11.36$, $p < 0.01$; Figure 2.2b), and attacked the models more if the ASY model was presented first, although this latter relationship only approached significance ($F_{1, 11} = 4.49$, $p = 0.06$). No relationship was found for the number of resident male attacks on the models (ASY model: 1.26 ± 0.89 attacks; SY model: 0.07 ± 0.07 attacks; $F_{1, 13} = 2.05$, $p = 0.18$).

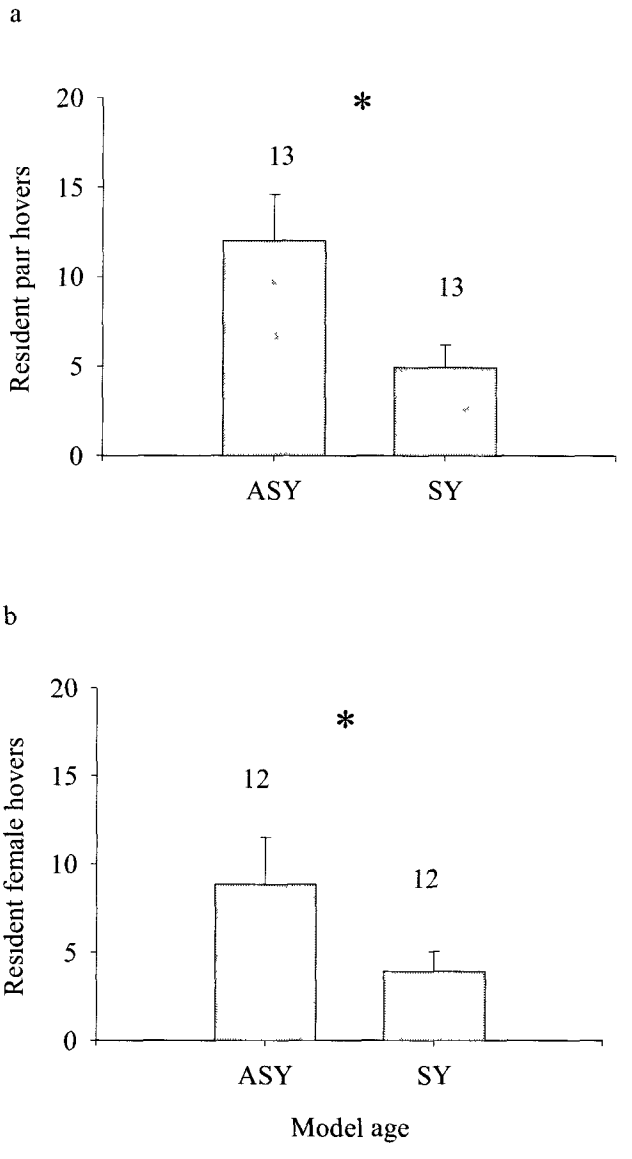

Figure 2.1 Mean number of hovers (± SE) by resident a) pairs, and b) females, over SY and ASY model female tree swallows. Models were separately placed directly on resident pairs' nest boxes to simulate an aggressive territorial intrusion. Sample sizes are indicated above error bars. Asterisks indicate significant differences at the $p = 0.05$ level.

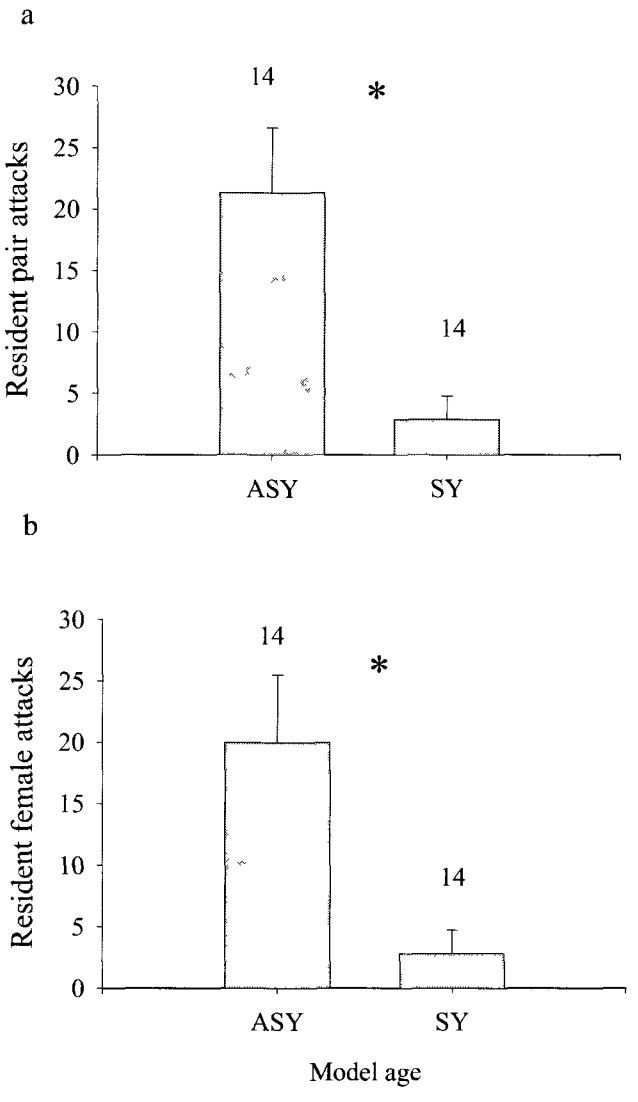

Figure 2.2 Mean number of attacks (± SE) by resident a) pairs, and b) females, on SY and ASY model female tree swallows separately placed directly on resident pairs' nest boxes to simulate an aggressive territorial intrusion. Sample sizes are indicated above error bars. Asterisks indicate significant differences at the $p = 0.05$ level.

2.5.2 Effects of model resident plumage colour on intruder behaviour

When examining the influence of resident plumage colour on intruder behaviour, I found that the ASY model elicited a higher number of scores that included attacks by intruding tree swallows compared to the SY model ($F_{1,25} = 25.3, p < 0.01$; Figure 2.3a) and elicited significantly higher mean intrusion scores ($F_{1,25} = 5.53, p < 0.01$; Figure 2.3b). When models were presented near neighboring nest sites at lower stages of nest construction, the models suffered more aggressive intrusions, as both of these intrusion measures were negatively related to the highest nest stage of the two closest neighboring nest sites (mean intrusion score: $F_{1,25} = 4.48, p = 0.04$; proportion of scores with attacks: $F_{1,25} = 6.07, p = 0.02$). I did not find any effects of the time or date of the presentation on the mean intrusion scores or the proportion of scores that included attacks on the models (all $p > 0.13$). Furthermore, I did not find any relationship between the age of the models and the proportion of scores that included nest box inspections ($F_{1,23} < 0.01, p = 0.99$), or the number of intrusions per minute ($F_{1,23} < 0.01, p = 0.99$).

2.6 Discussion

2.6.1 Model intruder plumage colour and resident female behaviour

When examining the influence of model plumage colour on behaviour of resident tree swallows, I found that resident females were significantly more aggressive towards the ASY model compared to the SY model (Figure 2.1b). My results differ from those of Stutchbury and Robertson (1987a), who found that resident females were equally aggressive to ASY and SY models, and may be due to territories in my population being easier for females to obtain and maintain. During the breeding season, unoccupied nest boxes are dispersed throughout

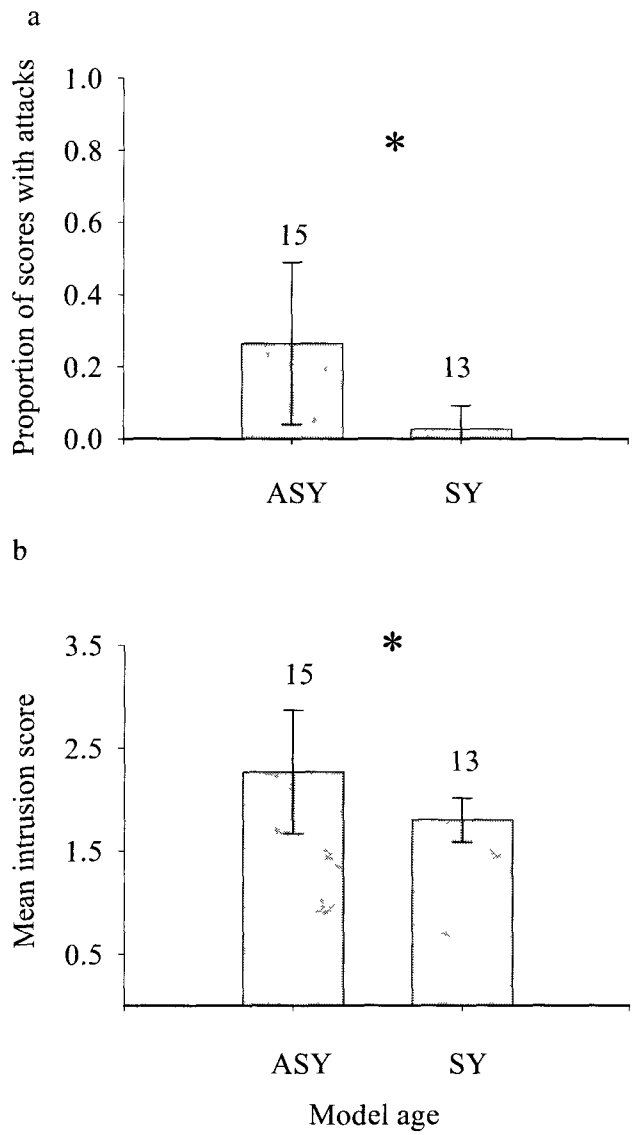

Figure 2.3 The mean (± SE) (a) proportion of intrusion scores with attacks and (b) intrusion score of tree swallows entering territories with either ASY or SY female models as territory holders. Asterisks indicate significant differences at the $p = 0.05$ level. Sample sizes are indicated over error bars. See Methods (section 2.3) for details on intrusion scores.

my study sites, with as many as 45% of the nest sites remaining vacant for the duration of the breeding season (O'Brien and Dawson 2007). Tree swallows prefer to nest no closer than 15m from their nearest neighbor, a tactic believed to reduce nest usurpation by neighboring conspecifics (Muldal et al. 1985). The increased distance from one occupied territory to another in my population may result in fewer intrusions into occupied territories and a reduction in the defensive behaviour of females at their nest sites during an intrusion, allowing any potential adaptive function of subadult plumage to be more evident with respect to territory establishment.

The aggressive behaviour of female tree swallows towards intruding females during the breeding season is believed to either be a means of securing a socially monogamous mate or to prevent territory eviction, neither of which is directly related to the quality of the nest site in terms of habitat or resources (Muldal et al. 1985). The aggressive behaviour of resident females towards the ASY model supports the hypothesis that ASY female tree swallows are perceived as greater territorial threats compared to SY females and therefore elicit more aggressive territorial defensive behaviour from resident females. In female collared flycatchers *(Ficedula albicollis*), wing patch size is an honest signal of age and body condition, and predicts the mating strategy of males (i.e. monogamy versus polygyny; Hegyi et al. 2008a). Recently, Hegyi et al. (2008b) found that when resident female collared flycatchers were presented with model females with varying wing patch size, resident females were more aggressive towards models with larger wing patch size. Since female plumage colour is an honest signal of age in tree swallows (Hussell 1983), resident females may be more aggressive when defending their nest site against individuals that are known to be older and are potentially more experienced competitors.

When models were presented on poles in front of the resident pairs' nest boxes, I found that SY resident females were significantly more aggressive towards the models compared to ASY resident females. In other species, less experienced breeders have been found to be much more aggressive territory defenders (Landmann and Kollinsky 1995; Schwartz et al. 2007), potentially due to their tenuous ownership of their territories compared to more experienced breeders. There were very few SY resident females (4 of 25) in this experiment, however, and while more SYs were included in the second experiment where models were presented on nest boxes (6 of 14), resident female age was not a factor in their behaviour towards the models. Further investigation is therefore required to determine how age of resident females influences their response to intrusions within their territories.

I also found that resident females that occupied nests in earlier stages of nest construction were more aggressive towards the ASY model intruder. In tree swallows (Whittingham et al.1994) and other species (e.g. Sandell and Smith 1997) where females are competing for male-occupied territories, as opposed to territories alone, resident females have been found to be more aggressive towards intruders during the pre-laying and laying stages of reproduction compared to later in the breeding season. Resident females are believed to be more territorial at this time to prevent nest usurpation from intruding females and to secure their occupancy at their nest site, prior to the pair's further investment in reproduction (i.e. egg laying and nestling stages). If females at earlier nest stages are less secure of their occupancy at a nest site, it would seem reasonable for those females to be more aggressive toward any intruders that could potentially usurp their nest site compared to a female that has spent more time securing the pair bond with her mate.

When models were presented directly on resident pairs' nest boxes and the ASY model was presented first, the increased activity (i.e. hovers and attacks) over the models, in particular by resident females, may indicate that resident females were in an increased state of defensive behaviour after interacting with the ASY model. Testosterone levels have been found to increase in birds within 10 minutes after a simulated territorial intrusion (Wingfield and Wada 1989). If resident females were experiencing elevated hormonal levels after interacting with the ASY model, this may have resulted in continued aggression towards the SY model after the ASY model presentation (Wingfield et al. 1987). As I did not measure the testosterone profiles of residents, the hormonal response of tree swallows during agonistic interactions is worthy of further investigation.

2.6.2 Model intruder plumage colour and resident male behaviour

In contrast to Stutchbury and Robertson (1987a), I did not find a significant effect of model plumage colour on behaviour of resident males. I was unable to determine the sex of residents during some observations, and the possibility that males were more aggressive towards one of the models therefore cannot be eliminated. Nonetheless, non-aggressive behaviour of resident males toward the models may be because males are able to easily distinguish ASY females from males based solely on plumage characteristics, and were therefore not threatened when the models were presented as intruders. Although the plumage colour of ASY female tree swallows is very similar to that of males, females generally have more greenish-coloured upperparts and sooty grey-white breast plumage compared to males (Cohen 1984b). If male tree swallows are able to distinguish ASY females from males, this would reduce some of the costs associated with territorial defense for males, such as

increased risk of injury (Stutchbury 1992) or energy depletion (Studd and Robertson 1988) and increase time spent on beneficial activities (i.e. copulating, feeding) (Stutchbury 1992).

Stutchbury and Robertson's (1987b) population had a large number of floating females that occupied newly vacant nest boxes within a matter of 10-15 minutes after removal of the resident female. Their population also has floating males, indicating limited breeding opportunities for both sexes, and an increased potential for heightened territorial behaviour of males in their population (Stutchbury and Robertson 1987b). The males in Stutchbury and Robertson's population may be highly sensitive to any intruders, regardless of their sex. If so, a male's immediate aggressive response to intruders may increase his chances of remaining at that nest site (Parker 1976) and would outweigh the costs associated with increased territorial aggression in birds (Studd and Robertson 1988; Stutchbury 1992). Furthermore, Leffelaar and Robertson (1985) found that when female tree swallows intruded into occupied territories, males would not equally participate in territory defense and would, at times, attempt to copulate with the intruding females. Although socially monogamous, tree swallows engage in extra-pair copulations (Lifjeld et al. 1993; O'Brien and Dawson 2007), and a male's lack of defensive behaviour at his nest site, as seen in my experiments, may indicate his interest in gaining an extra-pair mate or a new social mate, further supporting a role for female aggression in this species.

2.6.3 Model resident plumage colour and intruder perception of models

When examining the influence of plumage colour of territory owners on intruder behaviour, I found that the ASY model suffered significantly more aggressive intrusions compared to the SY model (Figures 2.3 a,b). I also found, however, higher mean intrusion scores and a higher

proportion of scores that included attacks on the models when model territories were situated near neighboring tree swallows at lower stages of nest building. As I found that the stage of nest construction was related to the amount of resident aggression in my experiments where models were presented as intruders, the tree swallows that entered the model territories may have been neighboring birds defending their own territories as opposed to birds actively searching for nest sites. Similar to Stutchbury and Robertson's (1987a) results, mine are confounded by the fact that I was unable to identify the sex of all intruding tree swallows due to multiple birds simultaneously entering my model territories. Regardless, this does not diminish the fact that the ASY model received much more aggressive encounters compared to the SY model.

Overall, my findings suggest that subadult plumage is beneficial to SY female tree swallows by reducing aggression from conspecific females during the breeding season. If this is true, it is unclear why females moult into the iridescent blue-green plumage after their first breeding season, when it elicits a much more aggressive response from conspecifics. ASY female tree swallows with bluer plumage have been found to have higher fledging success and assortatively mate with males based on plumage brightness (Bitton et al. 2008). If males are adjusting their reproductive effort based on their assessment of female quality from plumage characteristics, this may indicate an adaptive function of plumage colouration in ASY females. Further studies should examine the influence of female plumage colouration in terms of territory acquisition and status signaling as well as the influence of plumage colouration with respect to sexual selection using mate-choice experiments.

3. Variation in subadult plumage reflectance of female tree swallows predicts reproductive success

3.1 Abstract

Tree swallows are one of two species of North American passerines where females, rather than males, exhibit delayed plumage maturation, and display a distinct subadult plumage in their second year of life (SY). Studies suggest that this brown, subadult plumage of SY females may reduce aggression from conspecifics during the breeding season. There is, however, a great deal of variation in the amount of iridescent blue-green, adult-coloured plumage displayed by SY females, suggesting this plumage trait may be condition-dependent and hence an indicator of quality. To date, the reflective properties of the plumage of SY female tree swallows have not been reported; therefore I measured the spectral reflectance of the plumage of SY females and related variation in this trait to female condition and reproductive success. I predicted that SY females with more adult-coloured, iridescent blue-green plumage would have higher reproductive success, as plumage colouration has been found to be related to reproductive success in older females that are after their second year of life (ASY). In contrast to my expectations, SY females with browner plumage were in better body condition compared to more iridescent blue-green SY females and were mated to males that had higher UV-blue reflective plumage, suggesting assortative mating for plumage colouration. Browner SY females also had more feathers lining their nests, which may suggest that males are adjusting their reproductive investment based on SY female plumage characteristics. Furthermore, offspring raised by browner SY females had faster structural and feather growth and were larger at fledging. I propose that increased agonistic encounters

may account for the reduced reproductive success and condition of more iridescent blue-green SY females in my study and may be a selective force shaping the evolution of DPM in this species.

3.2 Introduction

The signaling function of plumage ornaments has been extensively studied in male birds, yet examination of a communicative role of female plumage ornaments is far less common. Female plumage ornaments have been less studied potentially due to the assumption that they are merely genetic correlates of male ornaments (Lande 1980), or that there is little competition between females to attract mates as conventional ideas about sex roles regard the female as the choosier sex (Andersson 1994). On the contrary, evidence is accruing that some female ornaments have evolved independently of male phenotypes (Price and Birch 1996) and are indicators of quality (Roulin et al. 2001; Siefferman and Hill 2005a), social status (Murphy et al. 2009), and less-commonly, age (Stutchbury and Robertson 1987a; Thompson and Leu 1995).

Much research is currently focused on plumage colouration being a condition-dependent signal of quality, where quality may entail aspects such as the bearer's physical condition, reproductive capabilities or genetic characteristics (Norris 1993; Siefferman and Hill 2003). Condition-dependent traits are considered so because differential costs, such as energetic or social costs (Hamilton and Zuk 1982; Kodric-Brown and Brown 1984), are associated with the acquisition and maintenance of these traits to ensure the signaler is communicating an "honest" signal to the potential receiver(s). Costs associated with plumage colouration also can vary depending on the mechanism of colour production. Melanin

pigments are believed to be less sensitive to individual condition (McGraw et al. 2002) compared to carotenoid- and structural- based colours, which have commonly been found to be costly to produce and are indicative of quality (Hill and Montgomerie 1994; Keyser and Hill 1999). Melanin plumage colouration is determined by the molecular structure of the pigment molecule and its concentration within the feather. Structural colouration, however, is more complex and is a consequence of the size, spatial distribution and refractive indices of different combinations of nanostructures such as pigment molecules, air and keratin within the feather (Prum 2006). The mechanisms driving the condition-dependence of structural colouration are largely unknown, although developmental perturbations may cause variation in the number or thickness of these feather nanostructures and could affect the brightness and colour of the feather (Prum 2006). If these nanostructures are costly to produce, as has been suggested (Shawkey et al. 2003; Doucet et al. 2006), this may be the physiological mechanism allowing structural plumage colouration to be an honest indicator of individual quality. These differential costs of plumage colour production have enabled plumage colouration to be a predominant consequence of sexual selection in birds.

Although plumage colouration is commonly related to sexual signaling (Darwin 1871; Andersson 1994), it can also communicate social status in intrasexual interactions (Lyon and Montgomerie 1986). Delayed plumage maturation (DPM) is a phenomenon where there is a delay in the acquisition of adult plumage traits without a delay in sexual maturity, and is commonly exhibited by males (Rohwer and Butcher 1988). DPM can result in a subadult plumage that is often drabber than the adult-coloured plumage (Rohwer et al. 1980) and is mainly thought to be an honest signal of age or low competitive threat (reviewed in Senar 2006). From a breeding perspective, DPM occurs in species that have high levels of

intrasexual competition for mates and territories (Beauchamp 2003); therefore, drab subadult plumage is thought to reduce aggression from older conspecifics, allowing younger individuals easier access to mates (Lyon and Montgomerie 1986).

Tree swallows (*Tachycineta bicolor*) are one of only two species of North American passerines where females, rather than males, display DPM (Morton 1989). Females in their second year of life (SY) have dorsal plumage that is predominately a subdued, brown colour in comparison to the iridescent blue-green (adult-coloured) plumage of males and females that are after their second year of life (ASY) (Hussell 1983). Nonetheless, SY females can display variable numbers of iridescent blue-green feathers on their dorsal surface, and Hussell (1983) reported that SY females varied from completely brown to 50% iridescent blue-green upperparts, with rare instances of SY females displaying fully iridescent blue-green plumage.

The few empirical studies that have examined a potential communicative function of subadult plumage of SY female tree swallows have suggested it may reduce conspecific aggression during the breeding season by communicating subordinate status to ASY females (Lozano and Handford 1995) and female status to males (Stutchbury and Robertson 1987a). If this is true, SY females that exhibit the highest amount of DPM may be at an advantage compared to SY females that display more adult colouration. In other species, younger birds that exhibit more adult-like plumage experience increased aggression from older conspecifics, potentially because of their increased ability to attract mates (Hill 1989; Senar et al. 1998). SY female tree swallows that exhibit higher amounts of DPM may be displaying their age and inexperience more honestly to older individuals and therefore could face less harassment compared to more adult-coloured SY females. SY females with more DPM could

therefore have increased reproductive success if they are not targets of aggression from older individuals (Greene et al. 2000).

Alternatively, in species where SY males exhibit varying amounts of DPM, those that most resemble older individuals commonly have higher mating and breeding success compared to individuals with less adult-coloured plumage (Hill 1988; Grant 1990). This suggests that in addition to being a signal of age or social status in intrasexual interactions, DPM may also serve as an intersexual signal of individual quality. As signals of quality are known to have higher degrees of variability than non-signaling morphological traits (Møller and Hoglund 1991; Rowe and Houle 1996), the marked variation in plumage colouration within SY female tree swallows could be attributed to differences in individual quality. The reflective properties of the plumage of SY female tree swallows, however, have not been reported. Female tree swallows are highly aggressive during the breeding season (Leffelaar and Robertson 1985; CD Coady personal observation), and although female aggression has been found to be beneficial for acquiring nest sites and mates (Rosvall 2008), it would also seem important for SY females to communicate quality to potential mates. Males invest a large amount of time and energy in territorial defense (Robertson et al. 1992) and nestling provisioning (Leffelaar and Robertson 1986), corroborating a role for male mate choice in this species (Johnstone et al. 1996). A correlative study by Bitton et al. (2008) found that ASY female tree swallows mated assortatively with males for plumage brightness, and bluer, brighter females had higher reproductive success, suggesting that plumage colouration is an indicator of female quality and may influence male mate choice or parental investment in tree swallows. The brown colouration of feathers displayed by SY female tree swallows are produced via melanin pigmentation (Amundsen and Pärn 2006), while the iridescent blue-

green colouration of feathers is structural colouration produced via the feather nanostructure (Osorio and Ham 2002). If the variation in the amount of brown and iridescent blue-green plumage of SY females is due to physiological constraints (i.e. hormonal, energetic; Rohwer and Butcher 1988; Enstrom 1992) inhibiting the development of fully iridescent blue-green plumage, then perhaps only SY females that are of high-quality or in good condition may be able to produce the potentially costlier structural plumage colouration.

In this study, my intent was to investigate whether the plumage of SY female tree swallows is an indicator of quality by relating measures of reproductive success and reproductive investment of these females to the reflective properties of their plumage. I also examined whether the plumage of SY females was related to the reproductive investment of their mates. The differential allocation hypothesis posits that in species with biparental care, an individual's investment in reproduction will vary in relation to the attractiveness of their mate (Burley 1986; Torres and Velando 2005). Male tree swallows may therefore increase their reproductive investment when mated to more iridescent blue-green SY females if males are assessing female quality based on plumage characteristics. On the other hand, DPM has been found to be beneficial to SY females by reducing aggression from males (Stutchbury and Robertson 1987a) and females (Lozano and Handford 1995; Chapter 2) during the breeding season. Perhaps there are social consequences of displaying more iridescent blue-green plumage for SY females (Senar et al. 1998; Greene et al. 2000). If iridescent blue-green plumage is a signal of breeding experience in female tree swallows, this plumage colouration may elicit more aggression from ASY females and may represent an evolutionary motivation for the maintenance of DPM in SY female tree swallows.

3.3 Methods

3.3.1 Study area and field procedures

During the 2008 and 2009 breeding seasons I studied tree swallows breeding in nest boxes at four sites within a 25 km radius of Prince George, British Columbia, Canada. Study sites consist of open agricultural fields mixed with patches of coniferous and deciduous forest, and small wetlands. I visited nests every 1–3 days beginning in mid-May and subsequently documented the date that the first egg was laid and the clutch size. I placed temperature data loggers (Onset Computer Corp., Bourne, Massachusetts, USA) in a sample of nests (n = 18) at two different sites to document incubation behavior, which in birds may be costly and a good indicator of reproductive investment (Reid et al. 2002). I placed temperature probes in the middle of the clutch, slightly above the nesting material to measure the temperature regimes experienced by eggs during incubation. Temperature was recorded every 1 min from 5 days in 2008 and 4 days in 2009 after clutch completion until eggs hatched. I calculated the average temperature during incubation and the proportion of time spent off the nest using the software programs Raven and Rhythm (Cooper and Mills 2005). The accuracy of female incubation behavior was confirmed with direct observations of the nests.

Hatching success was calculated as the number of eggs that hatched relative to the total clutch size. Once hatching was complete, SY females and their mates were captured using swing-door traps. Females were aged as SYs from previous banding records, or according to plumage characteristics. Although SY females have been found to possess greater than 50% iridescent blue-green feathers on their upperparts, I took a conservative approach and only classified females of unknown age as SY if they had less than 50% iridescent blue-green upperparts (Hussell 1983). I measured adult body mass with a spring

balance (nearest 0.25 g) and combined length of the head and bill with digital calipers (nearest 0.1 mm). The lengths of the wing chord, ninth primary feather, outer rectrix feather and tail were measured with a ruler (nearest 0.5 mm). To obtain measures of adult body condition, I scaled body mass to body size by calculating residuals from linear regressions using size measures that showed the strongest relationship with mass for each sex. I used tail length for all captured females in my population ($F_{1, 153} = 5.48, p = 0.02$) and wing chord length for all captured males ($F_{1, 138} = 15.58, p < 0.01$). Nestlings were weighed to the nearest 0.125 g with a spring balance, and length of the head and bill was measured with digital calipers (nearest 0.1 mm) every 2 days from 4 to 16 days old. Nestlings fledge between 18 and 22 days of age (Robertson et al. 1992). The length of the right ninth primary feather, which begins growing around 6 days of age, was measured to the nearest 0.5 mm every 2 days from 8 to 16 days old. Growth rate constants of nestlings were estimated using logistic models for body mass and head-bill size, while a linear model was used for ninth primary feathers (Dawson et al. 2005a). As nestlings within a nest are not independent, I subsequently calculated mean growth rates in each nest for these variables. I used measurements taken when nestlings were 16 days old to estimate mass and size at fledging. Fledging success was calculated as the number of nestlings that successfully fledged relative to the total number of eggs that hatched.

To investigate the parental effort of breeding pairs, I observed nestling provisioning rates of males and females in a subset of nests ($n = 8$) when nestlings were 5 and 12 days old. Observations were performed between 9:00 to 16:00 at one site using binoculars from a distance of approximately 50 m from the nest box and were dictated into digital recorders and later transcribed. Observers recorded the sex of the adults and documented when they

entered and left the nest box to quantify the number of visits by each adult at a nest site. Each visit was considered to be a food delivery visit, as previous research has found visit frequency to be an accurate measure of feeding rates and parental care in this species (McCarty 2002).

As an additional measure of male investment, I quantified the number and mass of feathers brought to the nest, as male tree swallows bring most of the feathers to line the nest and will often fight with other males for feathers (Cohen 1985). Feathers are hypothesized to primarily function as insulation and can enhance nestling growth and so may be an important component of male reproductive success (Winkler 1993). Feathers were removed from a subset of nests (n = 12) at the end of the 2009 breeding season and were counted and weighed with an electronic balance to the nearest 0.01 g.

3.3.2 Plumage analysis

I collected back and rump feathers from both sexes and stored the samples in opaque envelopes at room temperature until spectral analysis. To determine spectral characteristics, four feathers from the same body area and individual were mounted to a piece of cardboard in an overlapping fashion to reproduce how they would naturally lay on a bird. The mounted feathers were placed on a flat, non-reflective black surface and reflectance was assessed using an Ocean Optics USB2000 spectrometer (Dunedin, Florida, USA) with a deuterium tungsten halogen light source (Avantes, Broomfield, Colorado, USA). I used a bifurcated probe held at a 90° angle from the feather surface in a cylindrical sheath to exclude ambient light. I took readings at three random locations on the areas of the feather that would be exposed and visible to other birds and recorded spectral data as the proportion of light

reflected every 0.37 nm between 300-700 nm relative to the reflectance of a WS-1 diffuse reflectance white standard. I used average values from the three readings to calculate spectral curves for each body region and computed measures of colouration using CLR: Colour Analysis Programs v.1.05 (Montgomerie 2008). For SY females I quantified measures of brightness, hue, UV chroma, blue chroma and 'brown' chroma to encompass all possible variation in plumage reflectance for these females. I calculated average brightness as the average percent reflectance between 300 nm and 700 nm ($R_{300\text{-}700\ nm}$) (Siefferman and Hill 2005a) and hue was calculated as the wavelength at maximum reflectance (R_{max}). I calculated UV chroma and blue chroma as the relative contribution of each range to the total brightness (UV chroma: $R_{300-400\ nm}/R_{300-700\ nm}$, blue chroma: $R_{400-510\ nm}/R_{300\text{-}700\ nm}$). I described 'brown' chroma as the relative contribution of the green to red colour range ($R_{510\text{-}700\ nm}/R_{300\text{-}700\ nm}$). This colour range has previously been used as a measure of 'brownness' in birds (Siefferman and Hill 2003) and I also used this range to account for the green sheen that can occur on the brown feathers of SY female tree swallows (Hussell 1983; CD Coady personal observation). For males I quantified measures of brightness, hue, UV chroma and blue chroma (Bitton et al. 2007). For males and females I used the average values for both body regions for all plumage reflectance variables to describe the plumage reflectance of the entire upper body region for each bird (Doucet 2002). Separately for males and females, I entered the variables into principal components analysis (PCA) to eliminate the multiple correlations among the measures. For SY females, the first component (PC1) explained 52.9% of the total variation and was negatively weighted by blue chroma, and positively weighted by hue and 'brown' chroma but very little by brightness and UV chroma (Table 3.1) and was therefore considered a measure of SY female 'brownness' and lack of iridescent blue-green

Table 3.1 Weightings of colour variables for SY female and male tree swallows derived from principal components analyses.

SY female plumage variables	PC1 (plumage 'brownness')
Hue (wavelength at max. reflectance)	0.92
'Brown' chroma (510-700nm)	0.91
UV chroma (300-400nm)	-0.08
Blue chroma (400-510nm)	-0.98
Mean brightness (average percent reflectance between 300-700nm)	0.09
Male plumage variables	**PC1 (UV-blue reflectance)**
Hue (wavelength at max. reflectance)	-0.97
UV chroma (300-400nm)	0.87
Blue chroma (400-510nm)	0.70
Mean brightness (average percent reflectance between 300-700nm)	-0.27

colouration (Figure 3.1). The second component extracted from my PCA was interpreted as capturing variation in UV chroma and brightness, however, my intent was to examine the relationship between the amount of DPM exhibited by SY females and reproductive success, so I only used PC1 in analyses in this study. For males, PC1 explained 56.8% of the total variation and was negatively weighted by hue and positively weighted by UV chroma and blue chroma (Table 3.1); PC1 was therefore considered a measure of blueness and UV-reflectance.

To validate my assumption that PC1 for SY females was a measure of brownness and less iridescent blue-green plumage, I photographed a small subset ($n = 9$) of the SY females in 2009 and quantified the amount of iridescent blue-green plumage on their upperparts (back and rump). SY females were held in a black cardboard box that excluded ambient light and the female's upperparts were placed against a window cut out of a divider within the box such that the entire upper part of the female filled the window. A photograph was taken of each female using the timer setting on a Canon Powershot G5 camera placed inside the box. Using Image J 1.42 (Rasband 2009), I outlined the iridescent blue-green portions of the upperparts and subsequently calculated the proportion of iridescent blue-green upperparts for these females. I quantified proportion of iridescent blue-green upperparts for each bird twice and finding my measures were highly repeatable (repeatability = 0.97, $F_{8,9} = 36.1$, $p = 0.0001$; Lessells and Boag 1987), used the average proportion of iridescent blue-green upperparts in analyses.

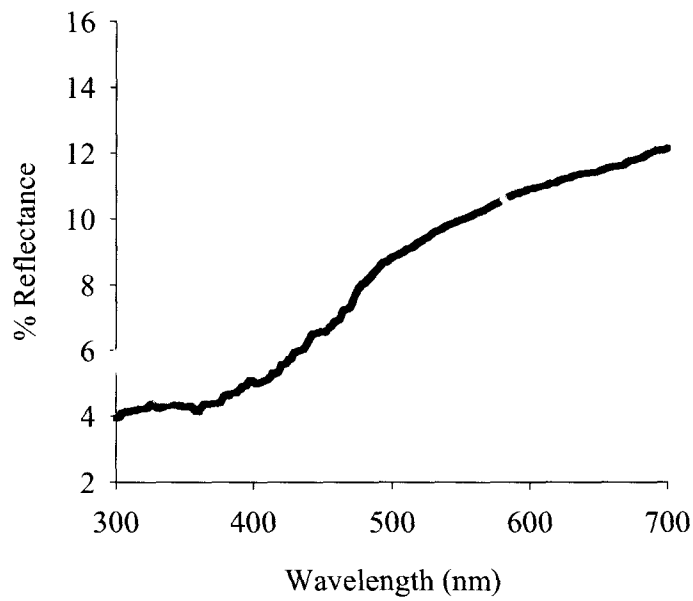

Figure 3.1 Spectral curves of SY female tree swallows with the highest (*black line*) and lowest (*gray line*) PC1 scores. PC1 is a measure of female 'brownness' and lack of iridescent blue-green (adult-coloured) plumage.

3.3.3 Statistical analyses

I used analysis of covariance (ANCOVA) to investigate if SY female plumage colouration predicted measures of female condition, reproductive investment (clutch initiation date, hatching success, brood size, fledging success, nestling growth rates and size at 16 days old) or predicted male quality (plumage colouration and body condition) and male reproductive investment (nestling provisioning rates, number and mass of nest feathers). Analyses also included year (2008 and 2009) as a categorical variable, and although the four field sites where data were collected are in close proximity, I also included site as a fixed factor. Female PC1 was used as a covariate in all analyses as a measure of female plumage colouration and male PC1 was used as a measure of male plumage colouration. To examine the proportion of time that females spent off the nest during incubation and the average egg temperatures during incubation, I also included clutch size as a covariate. For analyses of nestling growth rates and size, I included brood size and hatch date as covariates, which may influence nestling growth and condition. Provisioning rates were investigated by examining the number of visits to the nest by each sex separately both when nestlings were 5 and 12 days old. I included male and female body condition, brood size, the time of day that the observation was made and the partners' provisioning rates as covariates. Provisioning data were collected only in 2009 at one site, so year and site effects were not included in this analysis. Male reproductive investment was further investigated by examining the number and mass of feathers lining the nest. Feather mass was $\log_{10}$-transformed prior to analysis to meet the assumption of normality, and male condition was included as a covariate.

In all analyses I used a backward-stepwise procedure to eliminate terms and interactions that did not approach significance ($p > 0.10$), however, final models always

included the variable of interest (female PC1). All statistical analyses were performed using SPSS (Norušis 2000). Results were considered significant at the 0.05 level.

3.4 Results

I found that my measure of the proportion of iridescent blue-green plumage derived from photographs was negatively correlated with female PC1 (r = -0.65, p = 0.05, n = 9), supporting my use of PC1 as a measure of brownness and lack of iridescent blue-green plumage for SY females. I found that females that were browner (i.e. higher PC1 scores) were in better condition during the nestling period compared to females with more iridescent blue-green plumage ($F_{1, 60}$ = 4.8, p = 0.03; Figure 3.2). Female brownness was not related to clutch initiation date, the proportion of time spent away from the nest during incubation or the average egg temperatures during incubation (Table 3.2). I did not detect any effects of site or plumage brownness on brood size, hatching success or fledging success (all $p > 0.28$; Table 3.2). Female plumage brownness was not related to the number of provisioning visits to nest by females (Table 3.2), and the number of visits to the nest by males was also not related to the number of visits to the nest by females ($F_{1, 4}$ = 4.26, p = 0.11). Browner females raised young that grew ninth primary feathers faster than nestlings raised by more iridescent blue-green females. Browner females also raised young that were heavier, structurally larger (head-bill size) and had longer ninth primary feathers at 16 days old, just prior to fledging (Table 3.2). I found that browner females were mated to males with higher PC1 scores (i.e. higher UV-blue reflectance; $F_{1, 62}$ = 4.83, p = 0.03; Figure 3.3), yet female plumage brownness was not related to male condition ($F_{1, 64}$ = 1.1, p = 0.30). Browner females had a larger quantity ($F_{1, 10}$ = 8.46, p = 0.02, Figure 3.4) and a heavier mass

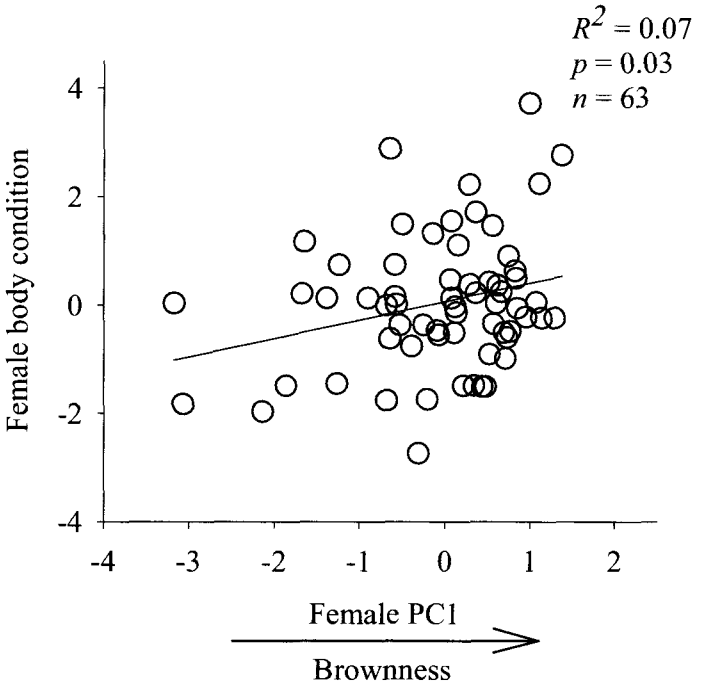

Figure 3.2 Relationship between plumage 'brownness' and body condition in SY female tree swallows. Body condition was estimated as the residuals from a linear regression of body mass on tail length.

Table 3.2 Relationship between PC1 ('brownness') of SY female tree swallows and reproduction.

Reproductive variable	Female plumage 'brownness' (PC1)				
	B	***SE***	***F***	***p***	***n***
clutch initiation date	0.32	1.05	0.09	0.76	79
clutch size	-0.02	0.09	0.06	0.81	79
hatching success	-0.03	0.04	0.61	0.44	67
ratio of time spent off the nest during incubation	0.00	0.01	0.35	0.56	18
average incubating egg temperature	-0.02	0.30	0.01	0.94	18
number of female nest visits during nestling stage	0.14	1.23	0.01	0.92	8
nestling head-bill growth rate	0.00	0.01	0.44	0.51	50
nestling 9th primary growth rate	0.20	0.08	6.57	**0.01**	49
nestling weight growth rate	-0.02	0.01	3.21	0.08	50
nestling head-bill size at 16 days old	0.21	0.09	4.87	**0.03**	50
nestling 9th primary length at 16 days old	2.33	0.01	9.51	**< 0.01**	49
nestling weight at 16 days old	0.61	0.07	5.13	**0.03**	50
fledging success	0.01	0.05	0.08	0.78	58

Hatching success is the number of eggs that hatched relative to the number of eggs laid per nest and fledging success is the number of nestlings that fledged relative to the number of eggs that hatched per nest Relationships significant at the $p = 0\ 05$ level have bolded p-values

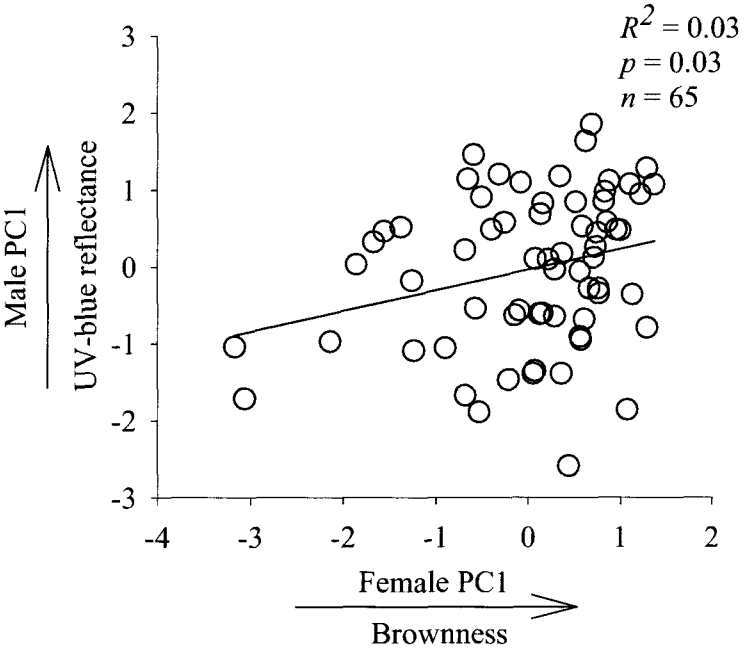

Figure 3.3 Relationship between plumage 'brownness' of SY female tree swallows and the UV-blue reflectiveness of their mates' plumage.

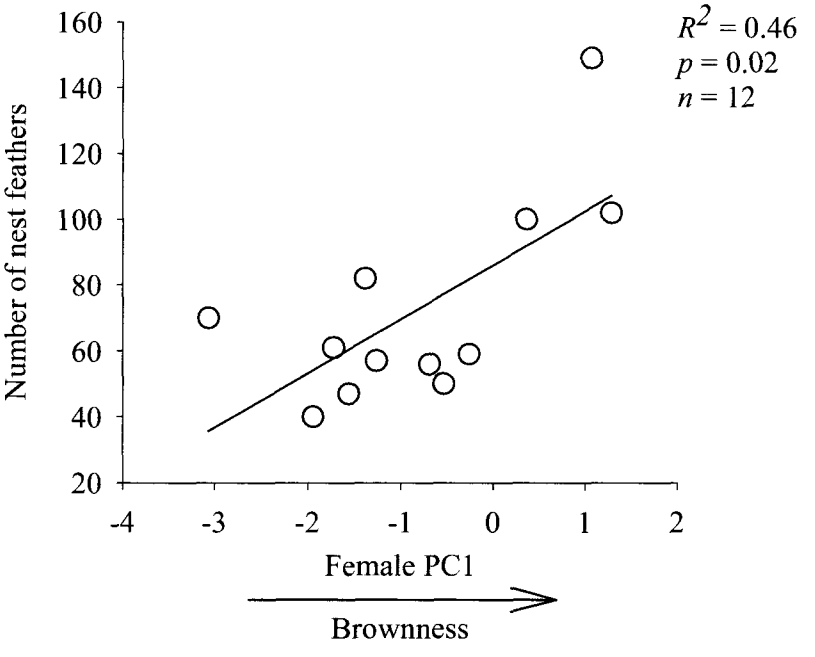

Figure 3.4 Relationship between plumage 'brownness' of SY female tree swallows and the number of feathers lining their nests.

($F_{1,10} = 5.08, p = 0.05$) of feathers lining their nests. Although these feathers are mainly brought by males, male condition and male plumage colouration were not significant (both $p > 0.45$) and did not remain in the model. Female plumage brownness was not related to the total number of provisioning visits to the nest by their mates ($F_{1,5} = 1.33, p = 0.30$).

3.5 Discussion

Contrary to my expectations, I found that males and SY females paired assortatively, although negatively, for plumage colouration (Figure 3.3) and that SY females with more iridescent blue-green upperparts faired poorer reproductively and were in poorer condition during the breeding season compared to SY females with browner plumage (Table 3.2; Figure 3.2). I cannot determine if the assortative mating for plumage characteristics is a result of mutual mate choice for plumage characteristics, as I did not perform mate choice experiments or manipulate plumage of either sex. If, however, mutual mate choice for plumage characteristics is occurring, that is, higher UV-blue reflective males are selecting browner SY females and vice versa, it seems contradictory that males would chose to mate with browner SY females when bluer plumage in ASY female tree swallows appears to be an indicator of quality (Bitton et al. 2008). Furthermore, if SY females with browner plumage are higher quality individuals, I would expect to see measures of maternal reproductive investment to be related to female plumage (Amundsen and Pärn 2006). On the contrary, I found that all measures of maternal investment in reproduction (i.e. clutch initiation date, clutch size, female time spent incubating, female nestling provisioning etc.) were unrelated to female plumage brownness (Table 3.2). These results suggest that plumage brownness is not an indicator of quality and is unlikely to be a sexually selected trait in this species. Instead,

males and SY females may be experiencing different selection pressures for these two opposing plumage types (Houtman and Falls 1994). If UV-blue reflectance is an indicator of quality in males and is selected by females, as has been found in other species (Keyser and Hill 2000; Balenger et al. 2009), browner females may be able to successfully pair with these potentially higher quality males if conspecific females perceive browner SY females as subordinate and less-threatening neighbors. In Lazuli buntings (*Passerina amoena*), for example, older males allow SYs with browner plumage to nest in close proximity, within high-quality territories, whereas older males are aggressive to intermediate-coloured SYs that are perceived as greater reproductive threats (Greene et al. 2000). In tree swallows, SY females with more iridescent blue-green plumage may be targets of intrasexual aggression and may be more frequently usurped from nest sites occupied by higher UV-blue reflective males.

Female tree swallows are highly aggressive and territorial during the breeding season, with intrasexual encounters often leading to injury, or in some cases death, in defense of suitable nest sites and mates (Leffelaar and Robertson 1985; CD Coady personal observation). Of the few studies that have examined a communicative function of subadult plumage in SY female tree swallows, results suggest that subadult plumage may function to reduce conspecific aggression by signaling female status to males (Stutchbury and Robertson 1987a) and subordinate status to females (Lozano and Handford, 1995; Chapter 2). If females with subadult plumage are perceived as less threatening to other females, the behavior of conspecifics may also depend on variation in this plumage trait, and SY females with higher amounts of iridescent blue-green plumage may experience more aggression from other females. In species where SYs have varying degrees of subadult plumage, individuals with

more adult-like plumage are commonly targets of aggression from older individuals (Hill 1989; Greene et al. 2000) and under certain conditions can have decreased body condition and increased mortality rates compared to birds with less adult-like plumage (Grant 1990; Senar et al. 1998). If more iridescent blue-green SY females are experiencing increased harassment from ASY females, this may account for the reduced reproductive success (Table 3.2) and body condition (Figure 3.2) of these females in my study.

I found more feathers lining the nests of browner females (Figure 3.4), which may indicate that their mates collected more feathers from the surrounding environment, a task that can be energetically expensive and potentially costly in terms of fitness in this species (Winkler 1993; Dawson et al. 2011). If the amount of iridescent blue-green plumage of SY females is indicative of their extra-pair mating behavior, their social mates may use plumage to assess this and adjust their reproductive investment accordingly. Kempenaers and others (1999) found that the probability of female tree swallows having at least one extra-pair young in their nest increased with the proportion of iridescent blue-green plumage of these females. As female tree swallows are able, to a large extent, control extra-pair copulations by actively selecting or rejecting extra-pair mates (Lifjeld and Robertson 1992), SY females with more iridescent blue-green plumage may be more likely to seek out more extra-pair copulations during the breeding season compared to browner females. Although previous work on tree swallows has found no differences in male parental care when males are uncertain of their paternity (Whittingham et al. 1994; Kempenaers et al. 1998), these studies did not examine the influence of female plumage colouration on male reproductive investment, making this possibility deserving of further investigation.

Although the proximate mechanisms underlying the acquisition of iridescent blue-green plumage in female tree swallows have not been reported, in SY male black redstarts (*Phoenicurus ochruros*), Schwarzova et al. (2010) found that individuals that moulted into fully adult-coloured plumage had significantly higher plasma testosterone levels during moult compared to individuals that displayed DPM and moulted into subadult-coloured plumage. Their finding suggests that a lack of testosterone was responsible for DPM. With respect to tree swallows, McCarty and Secord (2000) and later Stapleton et al. (2001) have speculated that female plumage colouration may be a hormone-dependent trait. If so, variation in the amount of iridescent blue-green plumage in SY female tree swallows could also be associated with variation in reproductive behavior. Experimentally increased testosterone levels have been found to decrease reproductive success and parental investment in females of other species (O'Neal et al. 2008; Veiga and Polo 2008), and reduce choosiness when selecting mates (McGlothlin et al. 2004). If more iridescent blue-green SY females have higher testosterone levels, they could be less-attentive at their own nests and less choosy when selecting their mates. This behaviour could account for the reduced reproductive success of more iridescent blue-green female tree swallows and the negative assortative mating for plumage characteristics if browner females are more selective when choosing their mates.

My results suggest that there may be inter- and intrasexual costs to displaying higher amounts of adult-coloured plumage in SY female tree swallows, as has been found in other species (Senar et al. 1998; Greene et al. 2000). Yet what SY females are potentially communicating and to which potential receiver(s) has not been tested here and needs to be identified. I suggest that future studies manipulate the plumage of SY females and measure

the behavior of the manipulated female's social mate and that of other females to determine if SY female plumage influences inter- and intrasexual behavior. Separately, it would also be advantageous to examine if SY females with varying amounts of iridescent blue-green plumage differ in their promiscuity or aggression during the breeding season.

4. Structural plumage colouration predicts level of nest parasitism and reproductive success in female tree swallows

4.1 Abstract

Life-history theory suggests that organisms will make resource-allocation decisions to maximize their fitness. The factors underlying life-history trade-offs are largely unknown, however, individual quality has often been implicated as a mediator of these trade-offs. Theory and empirical studies state that plumage ornamentation can honestly signal individual quality due to the costs associated with plumage production and maintenance, and although studies are commonly directed at male ornamentation, female ornamentation has recently been shown to have signaling potential. I investigated if the structural plumage colouration of female tree swallows (*Tachycineta bicolor*) is an indicator of quality and could be indicative of how an individual chooses to balance investment in self-maintenance and reproduction. To simulate the need for a life-history trade-off, I immunized females with sheep red blood cells (SRBC) to activate their humoural immune response and examined female investment in reproduction and self-maintenance compared to control females injected with only phosphate-buffered saline (PBS). Surprisingly, I found no differences in the immunological response of females injected with SRBC and PBS, hence, treatment did not influence female investment in reproduction or self-maintenance and potential reasons why this may have occurred are discussed. I did, however, find that females with more UV-blue colouration raised young that were smaller prior to fledging and females with more UV-blue and brighter plumage had more parasites in their nests. My results are contrary to current theory that predicts ornamentation should increase with individual quality and reproductive success. My

findings, however, may still agree with current knowledge on the signaling function of ornamentation in birds if females are experiencing costs associated with higher social status or sexual selection.

4.2 Introduction

Environmental and ecological conditions dictate the diversity of life-history traits seen among species (Saether 1988; Williams et al. 2010). Within species the physiological mechanisms regulating life-history decisions, however, appear to be highly variable (Gustaffson et al. 1994; Ardia and Clotfelter 2005; Monaghan et al. 2009) and are less understood (Zera and Harshman 2001). Trade-offs are a central concept in life-history theory and one such trade-off is an organism's decision to allocate resources to current reproduction or to survival and future reproduction (Williams 1966; Stearns 1992). In birds, studies have shown that reproductive activities such as production and incubation of eggs and feeding of offspring are costly and can compete for resources with other physiological processes such as maintenance of body condition and immune function (Ilmonen et al. 2000; Hanssen et al. 2005). If trade-offs result from resource-based constraints, higher quality individuals that can effectively obtain resources should exhibit few costs of reproduction and therefore will be less subjected to trade-offs among competing physiological processes (Zera and Harshman 2001). Individual quality has been characterized in many ways in birds, however, age, physiological condition, and the time of year that individuals begin breeding are common measures of quality and are often related to higher reproductive success (Pilz et al. 2002 and references therein). These measures of quality have been found to be mediating factors in

life-history trade-offs in birds (Gustaffson et al. 1994; Ardia 2005a; Ardia and Clotfleter 2007).

The colourful plumages of birds have been extensively studied as sexually selected traits (Hill 2006a; Senar 2006) and have been implicated as indicators of individual quality. Plumage characteristics may be indicative of quality due to the energetic and social costs of producing and maintaining more elaborate plumage ornaments, allowing only high-quality individuals to express these traits maximally (Zahavi 1975; Hamilton and Zuk 1982; Kodric-Brown and Brown 1984). Pigment-based colouration (i.e. carotenoids and melanins) is commonly studied for its signaling potential due to the physiological link between pigment-based colouration and individual condition (McGraw et al. 2001; Tschirrin et al. 2003; McGraw 2007; Bokony et al. 2008). Structural-based colouration results from the interaction of light with the feather nanostructure (Osorio and Ham 2002) and the physiological mechanisms regulating variation in structural plumage colouration are not well understood (Keyser and Hill 1999; Prum 2006). Developmental perturbations, however, may cause variation in the number or thickness of the feather nanostructures and could affect the brightness and colour of the feather (Prum 2006). In fact, studies have found that variation in the hue and brightness of structurally-coloured feathers is related to the nutritional status (Keyser and Hill 1999) and physiological condition of the bearer (Doucet and Montgomerie 2003). If these nanostructures are costly to produce (Shawkey et al. 2003; Doucet et al. 2006), this may be the physiological mechanism allowing structural plumage colouration to be an honest indicator of individual quality.

Tree swallows (*Tachycineta bicolor*) are one of only two species of North American passerines where females, rather than males, exhibit delayed plumage maturation (Morton

1989). Females in their second year of life (SY) display mainly melanin-based, brown feathers with varying numbers of structurally-coloured iridescent blue-green feathers on their dorsal surface (Hussell 1983). Although some brown feathers can also be present on the dorsal surface of older females (after-second year of life; ASY) (Hussell 1983), ASY females have primarily structural-based plumage colouration and display iridescent feathers on their upperparts that range from green to blue in colouration (Cohen 1984b). Bitton et al. (2008) recently found that UV-blue colouration increased with age in female tree swallows and that brighter, more UV-blue females had higher reproductive success and that pairs mated assortatively for plumage brightness. These results suggest that plumage reflectance in ASY females may honestly signal individual quality.

The main objective of this study was to determine if plumage characteristics signal female quality in tree swallows and are indicative of how females will choose to balance investment in self-maintenance and reproduction. Previous work on tree swallows found that individual quality, as measured by clutch initiation date, mediated trade-offs between investment in reproduction and immune defense (Ardia 2005a). Therefore, if female plumage reflectance is indicative of individual quality, variation in reflectance may also predict the degree to which individuals allocate their limited resources to costly physiological processes. Furthermore, any potential trade-offs between investment in self-maintenance and reproduction, and how female quality mediates this trade-off, may be more evident when females are under stressful conditions (Friedl and Edler 2005; O'Brien and Dawson 2008). Activation of the immune system has been shown to be costly and is thought to be a nutritionally demanding and stressful process that necessitates trade-offs with other

physiological processes such as somatic growth, reproduction and temperature regulation (Sheldon and Verhulst 1996).

In this study, I experimentally activated the specific humoral response of female tree swallows in an attempt to reveal the underlying mechanisms directing life-history trade-offs in this species. I quantified investment in self-maintenance by measuring the level of haemagglutination post-immunization and the change in female body mass before and after treatment. I predicted that UV-blue colouration and brightness would increase with measures of individual quality and would be related to female reproductive investment. If plumage reflectance is indicative of female quality in tree swallows, more ornamented (i.e. more UV-blue, brighter) females should lose less mass post-immunization, have a higher immunological response to SRBC and be able to invest more in reproduction, compared to less ornamented females. Furthermore, I investigated if male reproductive investment was related to female plumage characteristics, as males may differentially invest in reproduction if they are assessing female quality based on variation in female attributes (Burley 1986), showing further support for female plumage reflectance as an indicator of quality in this species.

4.3 Methods

4.3.1 Study area and field procedures

During 2008 and 2009, I studied tree swallows breeding in nest boxes at four sites within a 25 km radius of Prince George, B.C., Canada. Study sites were open agricultural fields mixed with patches of coniferous and deciduous forest, and small wetlands. I visited nests every other day beginning in early May and then daily once egg laying had commenced to determine clutch initiation date and clutch size. To investigate female investment in

incubation, I placed temperature data loggers (Onset Computer Corp., Bourne, Massachusetts, U.S.A.) in a sample of nests (n = 32) to document incubation behaviour, which in birds may be costly and a good indicator of reproductive investment (Reid et al. 2002). I placed temperature probes in the middle of the clutch, slightly above the nesting material to measure the temperature regimes experienced by eggs during incubation. Temperature was recorded every 1 min starting 5 days after clutch completion until eggs hatched in 2008 and 4 days after clutch completion until hatch in the 2009 breeding season. I calculated the average temperature during incubation, and using the software programs Raven and Rhythm (Cooper and Mills 2005), I determined the proportion of time spent off the nest. The accuracy of female incubation behaviour was confirmed with direct observations of the nests.

Clutches were checked for hatching 12 days after the date of clutch completion and I defined the day the first egg hatched as Day 0 of the nestling stage. Hatching success was calculated as the number of eggs that hatched relative to the total clutch size. Once hatching was complete, adults were captured using swing-door traps. I only examined ASY females in this study, and females were aged from previous banding records or according to plumage characteristics. Although ASY females have been found to possess as little as 50% iridescent blue-green feathers on their dorsal surface (Hussell 1983), I took a conservative approach and only classified females of unknown age as ASY if they had greater than 90% iridescent blue-green upperparts. I measured adult body mass with a spring balance (nearest 0.25 g) during the chick-rearing period for males, and on the eighth day of incubation and then again 8 days later for females to investigate the change in female body mass pre- and post-immunization (immunization details below). I measured the combined length of the head and bill with

digital calipers (nearest 0.1 mm) and the lengths of the wing chord, ninth primary feather, outer rectrix feather and tail with a ruler (nearest 0.5 mm). To obtain measures of adult body condition, I scaled body mass to body size by calculating residuals from linear regressions using size measures that showed the strongest relationship with mass for each sex. For females, I used the pre-immunization body mass to eliminate the possibility of my treatments influencing my measure of female body condition. I used tail length for all females in my population ($F_{1, 108} = 13.45, p < 0.001$) and wing chord length for males ($F_{1, 80} = 10.82, p = 0.01$). Nestlings were weighed to the nearest 0.125 g with a spring balance, and length of the head and bill was measured with digital calipers (nearest 0.1 mm) every 2 days from 4 to 16 days old. The length of the right ninth primary feather was measured to the nearest 0.5 mm every 2 days from 8 to 16 days old. Growth rate constants of nestlings were estimated using logistic models for body mass and head-bill size, while a linear model was used for ninth primary feathers (Dawson et al. 2005a). As nestlings within a nest are not independent, I subsequently calculated mean growth rates in each nest for these variables. I used measurements taken when nestlings were 16 days old to estimate mass and size at fledging. Fledging success was calculated as the number of nestlings that successfully fledged relative to the total number of eggs that hatched.

To investigate the parental effort of breeding pairs, I observed nestling provisioning rates of males and females in a subset of nests ($n = 10$) when nestlings were both 5 and 12 days old. Observations were performed between 9:00 to 16:00 hours, in 2009 only, using binoculars from a distance of approximately 50 m from the nest box and were dictated into digital recorders and later transcribed. Observers recorded the sex of the adults and documented when they entered and left the nest box to quantify the number of visits by each

adult at a nest site. Each visit was considered to be a food delivery visit, as previous research has found visit frequency to be an accurate measure of feeding rates and parental care in this species (McCarty 2002).

I also quantified the number of feathers brought to the nest as a measure of male investment, as male tree swallows bring most of the feathers to line the nest and will often fight with other males for feathers (Cohen 1985). Feathers are hypothesized to primarily function as insulation and can enhance nestling growth and so may be an important component of male reproductive success (Winkler 1993; Dawson et al. 2011). Feathers were removed from nests and counted at the end of the 2009 breeding season.

At the end of the 2008 and 2009 breeding seasons I counted the number of parasitic blow fly (*Protocalliphora* spp.) pupae and puparia within each nest. In the 2009 breeding season alone, I also quantified the level of mite (*Ornithonyssus sylvarium* and *Dermanyssus* spp.) infestation by visiting nests on Day 21 or 23 of the nestling stage, once all nestlings had fledged, or when nestlings were no longer alive in the nest. I assessed mite infestation by placing one hand on the back inside wall of the nest box for 10 seconds and then again placing one hand one the outside front wall, covering the nest hole for 10 seconds. The number of mites on the hand was estimated for both the inside and outside of the nest box on a scale of 0 to 4, where 0 = no mites, 1 = 1-10, 2 = 11-100, 3 = 101-1000, 4 = 1001-10000. This scoring system is similar to that of Owen et al. (2009), and has been found to be highly correlated with the actual number of mites in the nest (Møller 1991; Owen et al. 2009). I used the average mite score for the inside and outside of the nest box as a measure of mite infestation, and as *Ornithonyssus sylvarium* and *Dermanyssus* spp. can be equally detrimental to their hosts (reviewed in Proctor and Owens 2000), I did not distinguish

between the two species for my measure of mite infestation. Both blow flies and mites are haematophagous and feed on nestlings and can detrimentally affect their growth and fitness (Merino and Potti 1995; Simon et al. 2004)

4.3.2 Immunological assay

On the eighth day of the incubation period I captured females and drew blood from the brachial vein to determine pre-exposure levels of antibodies from 10 μL of plasma. Using a hemocytometer, I counted the number of sheep red blood cells (SRBC; Quad Five, Montana, USA) to calculate the volume of SRBC to add to my phosphate buffered saline (PBS; GIBCO Invitrogen #434267, Burlington, ON). I subsequently injected each bird intraperitoneally with 5×10^7 SRBC suspended in 100 μL of PBS (Ardia 2005a). I recaptured all individuals to draw blood to determine post-exposure primary antibody titres in plasma 8 days later, which is within the time frame of peak antibody production in free-living birds (Snoeijs et al. 2007) and has previously been performed for this species (Ardia 2005a, b). I centrifuged blood samples at 5000 rpm for 10 minutes on the day of sample collection and plasma samples were stored at – 20 ° C until analysis. Each plasma sample was assayed using a 10 μL plasma volume. Antibody titres were measured using a base-2 serial dilution haemagglutination assay conducted on 96-well microtitre plates (Matson et al. 2005). For each individual, I added 10 μL of PBS to each well along one column of a plate and added 10 μL of plasma to the first well and then serially diluted the plasma sample down the column. After each sample was serially diluted down a single column on a plate, I added 10 μL of a 2% suspension of SRBC in PBS to each well and incubated the plate at 37° Celsius for 90 minutes. Upon completion of incubation, I titled the long axis of the plate to 45° at room

temperature for 20 minutes and then scanned the plate using the positive transparency (top-lit) setting at 300 dpi of a flatbed scanner (HP Scanjet G4050, Mississauga, ON). From these scans, I blindly scored titres according to bird identity and titres were recorded as the negative log 2 of the highest dilution of plasma showing positive haemagglutination. For each plate I used anti-SRBC antibody from rabbits immunized with washed SRBC (Rockland #24148, Gilbertsville, PA) as a positive control and wells with no plasma (PBS and SRBC alone) were used as negative controls. For samples where there was enough plasma to run replicates, duplicates were highly repeatable (Lessells and Boag 1987) for pre-injection (r = 0.95, $F_{1, 40} = 8.49$, $p = 0.006$) and post-injection plasma samples ($r = 0.97$, $F_{1, 47} = 11.47$, p = 0.001). The inter-assay variation for agglutination calculated from positive control samples was 14.7%.

4.3.3 Plumage analysis

I collected back and rump feathers from females and stored the samples in opaque envelopes at room temperature until spectral analysis. To determine spectral characteristics, four feathers from the same body area and individual were mounted on a piece of cardboard in an overlapping fashion to reproduce how they would naturally lay on a bird. The mounted feathers were placed on a flat, non-reflective black surface and reflectance was assessed using an Ocean Optics USB2000 spectrometer (Dunedin, FL, U.S.A.) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO, U.S.A). I used a bifurcated probe held at a 90° angle from the feather surface in a cylindrical sheath to exclude ambient light. I took readings at three random locations on the feathers and recorded spectral data as the proportion of light reflected every 0.37 nm between 300-700 nm relative to the reflectance of

a WS-1 diffuse reflectance white standard (Ocean Optics). I used average values from the three readings to calculate spectral curves for each body region and computed measures of reflectance using CLR: Colour Analysis Programs v.1.05 (Montgomerie 2008). I quantified measures of brightness, hue, ultra-violet (UV) chroma and blue chroma, which have previously been used as measures of plumage reflectance for this species (Bitton et al. 2008). I calculated average brightness as the average percent reflectance between 300 nm and 700 nm ($R_{300\text{-}700\ nm}$) (Siefferman and Hill 2005a) and hue was calculated as the wavelength at maximum reflectance (R_{max}). I calculated UV chroma and blue chroma as the relative contribution of each range to the total brightness (UV chroma: $R_{300-400\ nm}/R_{300-700\ nm}$, blue chroma: $R_{400-510\ nm}/R_{300\text{-}700\ nm}$). I averaged the values for the two body regions for all plumage reflectance variables to describe the plumage reflectance of the entire upper body region for each bird (Doucet 2002). I entered the variables into a principal components analysis to eliminate the multiple correlations among the measures. The first principal component (PC1) explained 64.9% of the total variation and was weighted by hue, UV chroma and blue chroma and was therefore considered a measure of UV-blue colouration. PC2 explained 25.1% of the total variation and was mainly weighted by plumage brightness (Table 4.1).

4.3.4 Statistical analyses

As previous work has shown that UV-blue colouration in female tree swallows increases with age (Bitton et al. 2008), I wanted to confirm this in my study. Using analysis of variance (ANOVA), I included female age as a predictor variable and examined both female PC1 and PC2 separately as dependent variables. I included only females whose exact age was known

Table 4.1. Weightings (rotated matrix values) of colour variables for female tree swallows derived from principal components analyses. The first component (PC1) is considered a measure of UV-blue colouration while the second component (PC2) is considered a measure of plumage brightness.

ASY female plumage variables	PC1 (UV-blue colouration)	PC2 (brightness)
Hue (Wavelength at max. reflectance)	-0.98	-0.17
UV Chroma (300-400nm)	0.94	-0.01
Blue Chroma (400-510nm)	0.69	0.57
Mean Brightness (average percent reflectance between 300-700nm)	0.05	0.96

($n = 26$) by using only those females in my analyses that had either hatched on my study sites or were caught in their second year and were accurately aged from plumage characteristics (Hussell 1983). Furthermore, as any potential relationships with plumage colouration could be due to changes in plumage colouration with age, I included female age as a covariate in all analyses of reproductive variables. Female age, however, was only related to clutch initiation date and is therefore only discussed within that particular analysis (see 4.4 Results).

I used analysis of covariance (ANCOVA) to investigate if female plumage reflectance predicted measures of female quality (i.e., body condition, clutch initiation date), reproductive investment (i.e. time spent incubating and nestling provisioning rates) and reproductive success (i.e. hatching success, brood size, fledging success, nestling growth rates and size at 16 days old) or predicted male reproductive investment (i.e. nestling provisioning rates and the number of feathers lining their nests). All analyses included treatment (PBS-injected or SRBC-injected) as a categorical variable to determine if immunization with SRBC influenced any potential trade-offs between female investment in reproduction versus self-maintenance. Year (2008 and 2009) was included as a categorical variable, and PC1 and PC2 were included as covariates in all analyses as measures of female plumage reflectance. To satisfy assumptions of parametric analyses, I square-root transformed female body condition. I included the difference in female body mass before and after treatment, and the level of haemagglutination post-immunization as measures of investment in self-maintenance. When examining the proportion of time that females spent off the nest during incubation and the average egg temperatures during incubation, I also included clutch size as a covariate. When examining the "time to hatch" which was the number of days from the date of clutch completion to the day the first egg hatched, I included

clutch size, clutch initiation date, proportion of time spent off the nest and the average egg temperatures as covariates. For analyses of nestling growth rates and size at fledging, I included brood size and hatching date as covariates, as these may influence nestling growth and condition. Provisioning rates were investigated when nestlings were 5 and 12 days old by examining the average number of visits to the nest by each sex separately. When examining the provisioning rates of eachparent, I included adult body condition, brood size, the time of day of the observation and their partners' provisioning rates as covariates. Provisioning rates were only examined in 2009 so year effects were not included in this analysis. Male condition was included as a covariate in the analysis of the number of feathers lining the nest.

When examining the level of nest parasitism, I used the number of blow fly pupae and puparia collected per nestling and level of mite infestation as dependent variables. I included hatching date as a covariate in the analyses to control for potential seasonal variation in parasite abundance (Burtt et al. 1991). I also included brood size when examining the level of mite infestation, as the number of mites in a nest is positively correlated with brood size in tree swallows (Burtt et al. 1991). The number of blow fly pupae per nestling was square-root transformed and the level of mite infestation was Box-Cox transformed to meet assumptions of parametric analyses. Although parasites have been found to influence growth and size of nestlings and reproductive success in birds (Merino and Potti 1995; Simon et al. 2004), I only quantified levels of mite infestation during the 2009 breeding season (n = 31). Therefore, I specifically examined the influence of the level of mite infestation on nestling growth and size using 2009 data only, although I also analysed the entire data set without mite infestation as an explanatory variable (above). Furthermore, I found both the level of mite infestation in nests and the number of blow flies per nestling

were related to female plumage characteristics (see Results), and so it was not possible to include both parasitism and plumage variables as explanatory variables in the same analyses (Sokal and Rohlf 1981).

In all analyses I used a backward-stepwise procedure to eliminate terms and interactions that did not approach significance ($p > 0.10$), however, final models always included the variables of interest (female PC scores). All statistical analyses were performed using SPSS (Norušis 2000). Results were considered significant at the 0.05 level and means are presented ± standard error (SE).

4.4 Results

Unexpectedly, I did not find any differences in the level of haemagglutination for plasma samples of females injected with SRBC (0.61 ± 0.24) and those injected with PBS alone (0.51 ± 0.21; $F_{1, 24} = 0.08$, $p = 0.78$). The lack of a difference in immunological response between treatment groups most likely explains the lack of any treatment effects on female reproductive investment (incubation off-bouts, average egg temperatures or female provisioning rates; all $p > 0.15$), reproductive success (time to hatching, hatching success, nestling growth rates, nestling weights at 16 days old, fledging success, or my measures of nest parasitism; all $p > 0.17$) and investment in self-maintenance (change in female body mass, level of haemagglutination; both $p > 0.30$). Therefore treatment did not remain in any of the final models.

Using known-age birds, I found a relationship between female age and plumage colouration, where older females possessed more UV-blue colouration, however this result only approached significance (i.e. higher PC1; $F_{1, 24} = 4.06$, $p = 0.06$; Figure 4.1). I found no

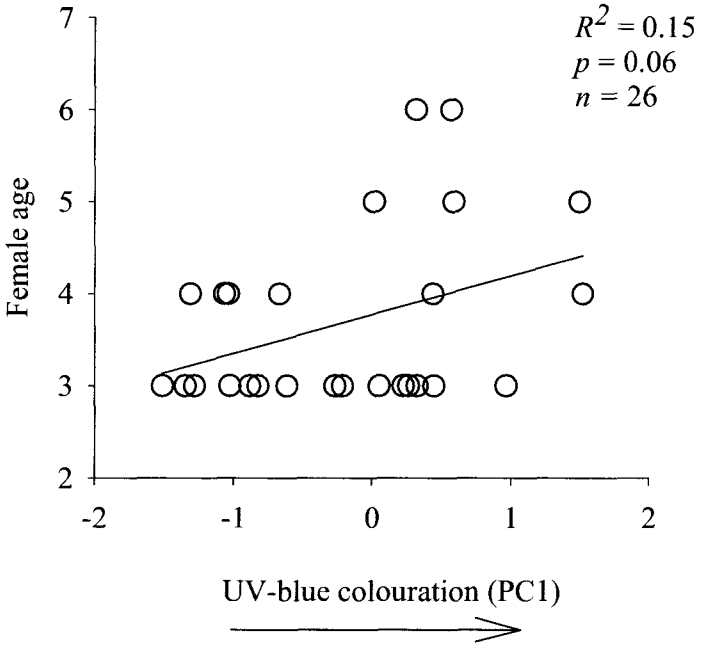

Figure 4.1 Relationship between age and UV-blue colouration (PC1) of female tree swallows. Only females of known age were included.

such relationship between female age and plumage brightness (i.e. higher PC2; $F_{1,24} = 0.51$, $p = 0.48$). Among all females, I examined the relationships between female plumage traits (PC1 and PC2) and measures of condition and reproductive success and investment. I found no relationship between plumage traits and body condition (PC1: $F_{1,73} = 1.08$, $p = 0.30$; PC2: $F_{1,73} = 0.23$, $p = 0.63$), although females in 2008 were in poorer condition ($F_{1,73} = 6.10$, $p = 0.02$). Female plumage traits were not related to the change in female body mass or the level of haemagglutination post-injection (both $p > 0.17$). There was no relationship between clutch initiation date and female plumage traits (PC1: $F_{1,78} = 0.98$, $p = 0.33$; PC2: $F_{1,78} = 0.53$, $p = 0.47$). Similarly, when I included female age in the analysis, I found that older females initiated their clutches earlier ($F_{1,22} = 5.83$, $p = 0.03$), but female plumage traits were also not related to clutch initiation date (PC1: $F_{1,22} = 0.64$, $p = 0.43$; PC2: $F_{1,22} = 0.13$, $p = 0.27$). I found no relationship between female plumage traits and clutch size (PC1: $F_{1,77} = 0.59$, p = 0.45; PC2: $F_{1,77} = 0.17$, $p = 0.68$). There was no relationship between female plumage traits and the proportion of time spent away from the nest during incubation (PC1: $F_{1,13} = 0.01$, $p = 0.99$; PC2: $F_{1,13} = 1.61$, $p = 0.28$) or average egg temperatures (PC1: $F_{1,13} = 0.17$, $p = 0.69$; PC2: $F_{1,13} = 0.46$, $p = 0.51$). I found that females with more UV-blue colouration took longer to hatch their young ($F_{1,75} = 5.87$, $p = 0.02$), whereas there was no relationship between time to hatch and plumage brightness ($F_{1,75} = 0.01$, $p = 0.97$). I found no relationship between hatching success and female plumage traits (PC1: $F_{1,68} = 1.25$, $p = 0.27$; PC2: $F_{1,68} = 0.70$, $p = 0.41$), although females in better condition had higher hatching success ($F_{1,68} = 5.95$, $p = 0.02$) and hatching success was higher in 2008 than in 2009 ($F_{1,68} = 5.77$, $p = 0.02$).

Female plumage traits were not related to the number of visits to the nest by females (PC1: $F_{1,7} = 0.80$, $p = 0.40$; PC2: $F_{1,7} = 0.14$, $p = 0.72$). I found no relationships between female plumage traits and growth rates of young (PC1: all $p > 0.31$; PC2: all $p > 0.16$). Ninth primary growth of young ($F_{1,66} = 19.04$, $p < 0.01$) and weight gain of young ($F_{1,67} = 6.85$, $p = 0.01$) were slower in 2009 compared to 2008, and ninth primary growth was also slower in larger broods ($F_{1,66} = 5.47$, $p = 0.02$). Mass of young at 16 days old tended to be higher in smaller broods ($F_{1,66} = 3.26$, $p = 0.08$) and decreased with female UV-blue colouration ($F_{1,66} = 3.95$, $p = 0.05$; Figure 4.2a), but was unrelated to female plumage brightness ($F_{1,66} = 1.19$, $p = 0.28$). I found that the length of the head-bill of young at 16 days old was larger in 2008 than in 2009 ($F_{1,66} = 8.27$, $p < 0.01$), and decreased with female UV-blue colouration ($F_{1,66} = 8.09$, $p < 0.01$; Figure 4.2b) but was unrelated to female plumage brightness ($F_{1,66} = 1.61$, $p = 0.21$). Ninth primary length of young at 16 days old was not related to any female plumage traits (PC1: $F_{1,65} = 0.97$, $p = 0.33$; PC2: $F_{1,65} = 2.90$, $p = 0.09$), although young at 16 days old had longer ninth primaries in 2008 compared to 2009 ($F_{1,65} = 17.31$, $p < 0.01$) and had longer ninth primaries in smaller broods ($F_{1,65} = 5.46$, $p = 0.02$). Neither UV-blue colouration ($F_{1,71} = 0.79$, $p = 0.38$) nor plumage brightness ($F_{1,71} = 0.40$, $p = 0.53$) was related to fledging success of young.

When I examined the relationship between female plumage traits, body condition and the level of parasitism in their nests, I found that the number of blow flies per nestling tended to increase with female UV-blue colouration ($F_{1,69} = 3.32$, $p = 0.07$) and significantly increased with female plumage brightness ($F_{1,69} = 8.15$, $p < 0.01$, Figure 4.3). I also found that females with higher amounts of UV-blue colouration ($F_{1,22} = 7.40$, $p = 0.01$, Figure 4.4a), brighter plumage ($F_{1,22} = 4.92$, $p = 0.04$, Figure 4.4b) and those in poorer condition

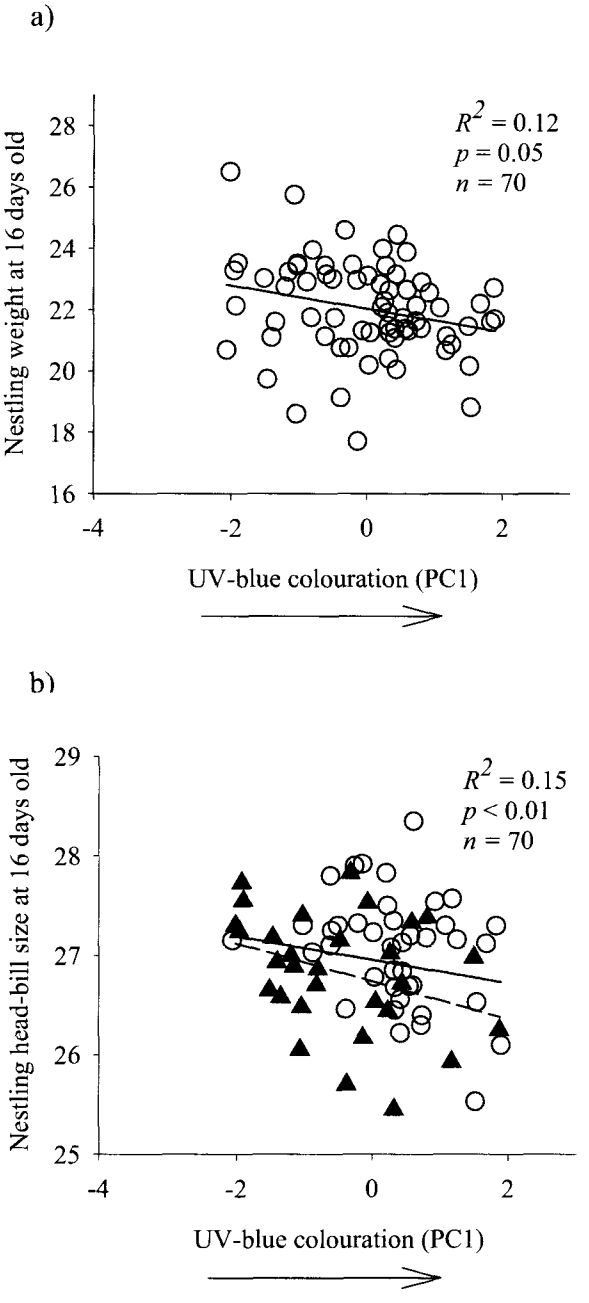

Figure 4.2 Relationship between (a) weight and (b) length of the head-bill of nestling tree swallows at 16 days old in 2008 (open circles and solid line, $n = 40$) and 2009 (shaded triangles and dashed line, $n = 30$), just prior to fledging and UV-blue colouration (PC1) of their female parent.

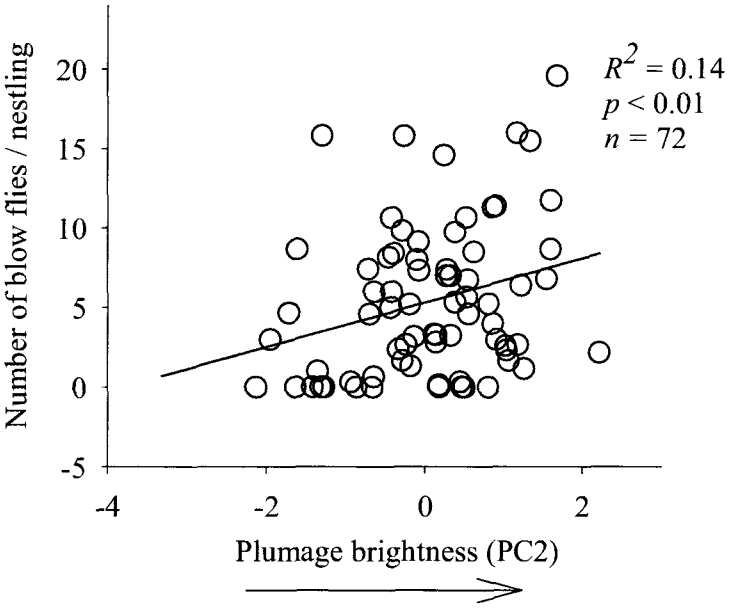

Figure 4.3 Relationship between number of blow flies (*Protocalliphora* spp.) per nestling and plumage brightness (PC2) of female tree swallows.

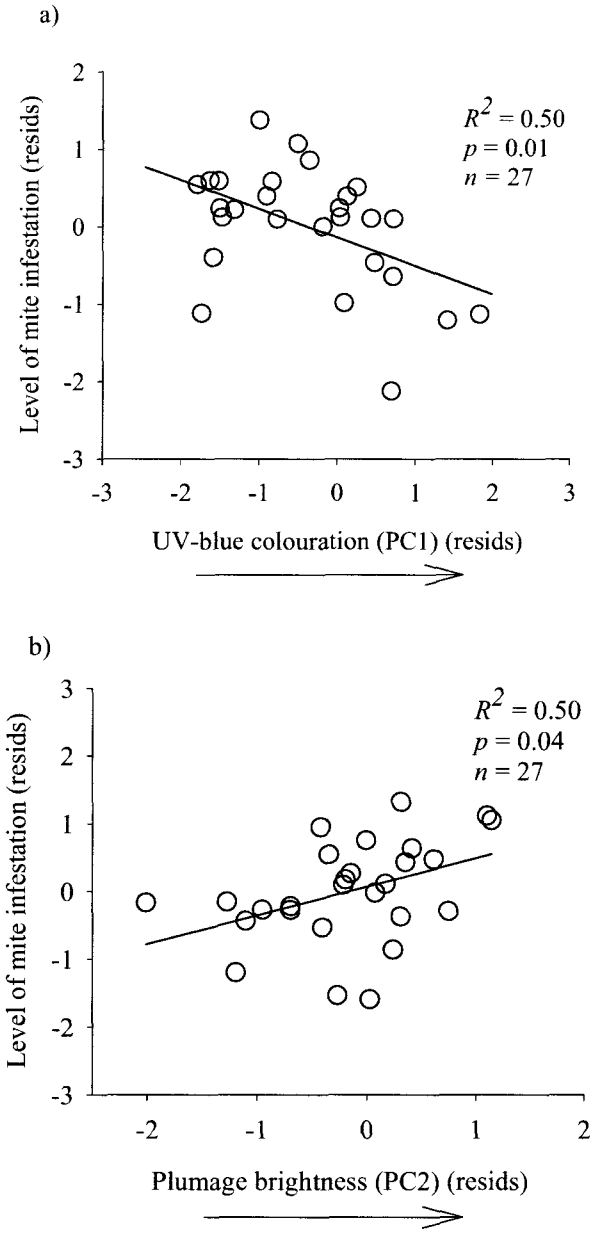

Figure 4.4 Relationship between level of mite infestation (*Ornithonyssus sylvarium* and *Dermanyssus* spp.) and (a) UV-blue plumage colouration (PC1) and (b) plumage brightness (PC2) of female tree swallows. Plots are residuals of the independent variable and the residuals of the dependent variable when both variables are regressed separately on the other independent variables in the model (see 4.4 Results).

($F_{1, 22} = 10.10, p < 0.01$) had higher levels of mite infestation in their nests, as did those nests that hatched later in the season ($F_{1, 22} = 9.40, p < 0.01$).

In separate analyses, I examined the influence of both measures of nest parasitism on growth rates and size of young at 16 days old, using 2009 data only when examining the level of mite infestation in nests. I found that the level of mite infestation did not influence head-bill growth ($F_{1, 27} = 1.04, p = 0.32$) or weight gain of young ($F_{1, 27} = 0.08, p = 0.78$), but I found a trend for higher levels of mite infestation to result in slower growth of ninth primary feathers of young, although this relationship only approached significance ($F_{1, 27} = 3.82, p = 0.06$). I found that higher levels of mite infestation resulted in smaller head-bill size ($F_{1, 26} = 5.51, p = 0.03$), and shorter ninth primary feathers ($F_{1, 26} = 11.27, p < 0.01$) of young at 16 days old, however, there was no relationship between the level of mite infestation and mass of young at 16 days old ($F_{1, 26} = 2.89, p = 0.10$). I found no significant relationships between the number of blow flies per young and the growth or size of young at 16 days old (all $p > 0.11$), however, young that were in nests with a higher number of blow flies per young had shorter ninth primary feathers at 16 days old ($F_{1, 67} = 3.84, p = 0.05$).

I also examined the relationship between female plumage traits and measures of male condition and reproductive investment. I found that female plumage traits were not related to the provisioning rates of their mates (PC1: $F_{1, 7} = 0.28, p = 0.61$; PC2: $F_{1, 7} = 0.92, p = 0.37$), although males that bred earlier in the season fed their young more often ($F_{1, 7} = 11.08, p = 0.01$). There was no relationship between female UV-blue colouration and the number of feathers lining their nests ($F_{1, 30} = 2.30, p = 0.14$), however, brighter females had fewer feathers lining their nests ($F_{1, 30} = 4.07, p = 0.05$; Figure 4.5). Although nest feathers

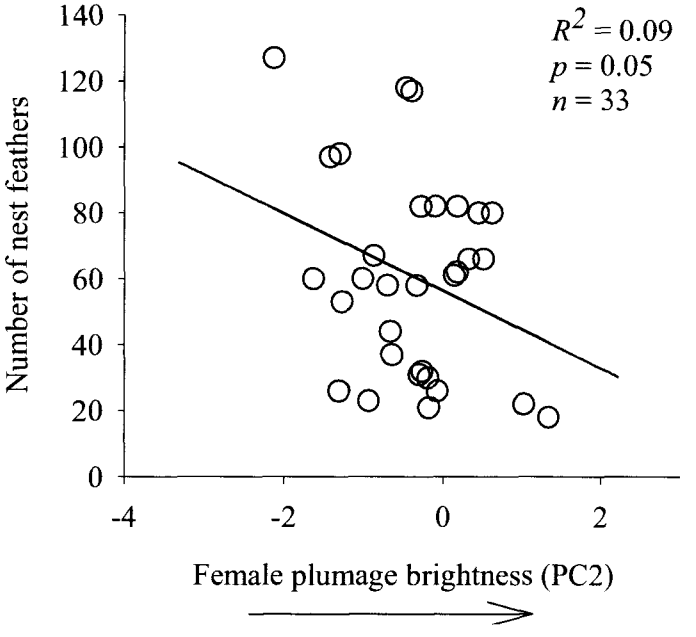

Figure 4.5 Relationship between number of feathers lining the nest and plumage brightness of female tree swallows (PC2).

are mainly brought by males (Cohen 1985), male body condition was not related to the number of feathers lining their nests and did not remain in the model ($p > 0.42$).

4.5 Discussion

Surprisingly, I did not find any differences in the immunological response of SRBC-injected and PBS-injected females, and hence did not find any influence of treatment on the magnitude of the potential trade-off between investment in reproduction and self-maintenance (Sheldon and Verhulst 1996; Deerenberg et al. 1997). SRBC injected into the peritoneal cavity of female tree swallows should have caused them to mount a primary humoural immune response resulting in the production of antibodies specifically directed against SRBC (Kuby 1997). The plasma of these females should have therefore contained antibodies specific to SRBC when I performed my blood sampling 8 days after injecting females, as this is believed to be a reliable time frame for measuring the primary immune response to SRBC in this species (Snoeijs et al. 2007) and has been successfully performed in other studies (Ardia 2005a,b). The positive agglutination results that I observed for both SRBC- and PBS-injected females may have instead been the result of natural antibodies (Nabs) binding to SRBC rather than antibodies specific to SRBC (Matson et al. 2005). Nabs are recognition molecules of the innate immune system and can be found in the body without any indication of infection (Abbas and Lichtman 2005). Furthermore, the response to SRBC by tree swallows has been found to vary between populations (Ardia 2005a) and individuals in my population may have lower to negligible responses to SRBC if they have differential exposure to parasites and pathogens thus affecting investment in immune function (Martin et al. 2004; Ardia 2005a). I also immunized females during the incubation stage of reproduction

when most studies have immunized females during the nestling stage of reproduction (Ardia 2005a, b). Studies indicate that incubation is much costlier than previously assumed (Heaney and Monaghan 1996; Williams 1996) and as female tree swallows are the only parent that incubate and are not fed by males during the incubation period (Kuerzi 1941), incubation may be a very energetically expensive task for females in this species. In other species, studies have found that individuals will suppress their specific immune responses when investing high amounts of energy into incubation and other costly stages of reproduction (Nordling et al. 1998; Hanssen et al. 2005). Female tree swallows may therefore also be experiencing immunosuppression during incubation if incubation is too energetically costly to allow females to invest in both incubation and the specific immune response.

My results indicate that females with more UV-blue colouration and brighter plumage had higher levels of mite infestation compared to females that were relatively greener and duller (Figures 4.4a,b). As mites can over-winter in nest boxes (Burtt et al. 1991), I also examined whether these results were due to the occupancy of the nest box during the prior breeding season. I found no relationship with the current level of mite infestation and prior documentation of mite infestation levels in nest boxes (Coady and Dawson unpublished data). The level of mite infestation in nests is therefore most likely attributable to transmission of mites from the adults nesting in those nest boxes rather than mites from previous breeding seasons (Møller 1991). As I found that the level of mite infestation increased with increasing amounts of female UV-blue colouration, plumage brightness (Figure 4.4a, b) and female body condition, and no such relationships with male phenotypic traits (Coady and Dawson unpublished data), the level of mite infestation found in my nests is most likely attributable to the level of mite infestation of adult females in my study. Since

females in poorer condition had higher levels of mites in their nests, these females may be more susceptible to parasitism if they do not possess or cannot obtain enough resources to mount an effective immune response against parasites (Goater and Holmes 1997), or they could be in poorer condition as a result of competition with the parasites for nutrients or energy resources (Dawson and Bortolotti 2000).

The relationship between female plumage traits and mite infestation was not due to females with higher amounts of UV-blue colouration and brighter plumage being in poorer condition, as I found no relationships between female body condition and plumage traits (see Results). Instead, females that display more UV-blue colouration and brighter plumage in my population may be more susceptible to parasites compared to females with greener, duller plumage due to different social pressures. I found a trend for UV-blue colouration to increase with female age (Figure 4.1), and previous research has found that female tree swallows in their third year of life had significantly less UV-blue colouration compared to older females (Bitton et al. 2008). I did not find any relationships between female age and parasitism (see 4.4 Results), however, age (Holberton et al. 1990) and UV-blue colouration and brightness (Reudink et al. 2009; Remy et al. 2010) have been found to be predictors of social status in birds. Therefore female tree swallows with more UV-blue colouration and brighter plumage could be of higher social status and may experience differential costs related to their dominant status.

Although it is commonly viewed that subordinate individuals have increased stress levels (reviewed in von Holst 1998), dominant individuals can also experience higher stress levels (Goymann and Wingfield 2004) and energetic costs (Jarvi and Bakken 1984) if their social status is repeatedly challenged. Chronically elevated levels of glucocorticoid hormones

can suppress the immune system (Owen-Ashley et al. 2001) and may influence the prevalence of parasites for dominant individuals if they do not possess the same immune defenses as less-stressed individuals (Poiani et al. 2000). Chronic stress levels and increased time spent in aggressive encounters could both result in decreased reproductive investment in bird species (Silverin 1986; Astheimer et al. 1992; Ellis and Good 2006) and could account for the smaller size of young raised by more UV-blue females in my study (Figure 4.2a, b). Furthermore, if more UV-blue and brighter females are dominant to greener, duller females, the time that these females spend in aggressive encounters with conspecifics could reduce their time available for self-maintenance activities, such as preening, which has been found to significantly reduce the number of ectoparasites on birds (Hart 1997). Weatherhead et al. (1993) found that male red-winged blackbirds (*Agelaius phoeniceus*) with longer red epaulets had higher levels of ectoparasites. Epaulet length is a potential correlate of dominance in red-winged blackbirds (Røskaft and Rohwer 1987) and thus may be an example of a cost to dominance in birds not normally discussed.

Although I did not examine nest site characteristics, more UV-blue and brighter females may possess territories with different characteristics compared to greener, duller females, if these plumage attributes are related to age, quality or dominance (Part 2001; Siefferman and Hill 2005b). Characteristics specific to the nest itself, such as microclimate (Dawson et al. 2005a) and nesting material (Gold and Dahlsten 1989; Heeb et al. 1996) can dramatically affect the survival and prevalence of nest parasites. If more UV-blue, brighter females are of higher social status they may have more opportunity to select their nest sites and could choose nest boxes with different microclimatic features (Ardia et al. 2006). More UV-blue, brighter females could be choosing nest boxes that provide an ideal microclimate

for rearing young, however, these same conditions could also be ideal for growth of blow fly larvae (Dawson et al. 2005a,b) resulting in more blow flies in the nests of these females (see 4.4 Results; Figure 4.4a,b). Furthermore, as nest architecture varies with age in this species (Lombardo 1994; CD Coady personal observation), more UV-blue females may build nests with different architectural features, which could account for the observed trend for more blow flies in their nests in my study (see Gold and Dahlsten 1989; Heeb et al. 1996). Bitton et al. (2008) implicated female UV-blue colouration and plumage brightness as potentially sexually selected traits in tree swallows as they found that pairs mated assortatively for plumage brightness and more UV-blue, brighter females had higher reproductive success. The plumage colouration of tree swallows is structural-based, and evidence is accruing for the condition-dependence and thus honest-signaling potential of structural plumage colouration in birds (Hill 2006b). To date, most studies examining structural plumage colouration have found that plumage brightness and UV-blue colouration are reduced in individuals that suffer from nutritional stress (Siefferman and Hill 2005a) and parasitic infection (Doucet and Montgomerie 2003), and that less colourful, duller individuals have lowered reproductive success (Siefferman and Hill 2005a; Doutrelant et al. 2008). I found that more UV-blue, brighter females had higher levels of parasitism (Figures 4.3 and 4a,b) and more UV-blue females faired poorer reproductively (Figure 4.2a,b), contrary to what would be expected if only high quality individuals can produce these potentially costly plumage traits (Zahavi 1975; Hamilton and Zuk 1982; Kodric-Brown and Brown 1984). If, however, UV-blue colouration and brightness are sexually selected traits, females that have more UV-blue, brighter plumage may experience increased social interactions with potential mates if they are perceived as higher quality and are thus more

attractive. Increased contact with potential mates (social or extra-pair mates) could result in an increased rate of transfer of parasites (i.e. mites) to more UV-blue, brighter females (Proctor and Owens 2000) resulting in the higher level of mite infestation observed in their nests. Burley et al. (1991) also found that more ornamented zebra finches (*Taeniopygia guttata*) had higher levels of parasites, which they speculated could have been a result of increased social contact with parasitized individuals.

If males perceive more UV-blue, brighter females as higher quality, I would have predicted that males mated to these females would invest more in reproduction (Burley 1986). Instead, I found that brighter females had fewer feathers in their nests, which may be indicative of male reproductive investment since the majority of these feathers are collected by males (Cohen 1985). It is unclear why brighter female tree swallows had fewer feathers in their nests and this finding warrants further examination.

Overall, my findings that UV-blue colouration and plumage brightness were related to reproductive success and parasitism in female tree swallows indicate that these plumage characteristics may signal some aspect of female quality or social status in tree swallows. My findings, however, are contradictory to what one would expect given the current models of sexual selection and status signaling in birds (Hamilton and Zuk 1982; Kodric-Brown and Brown 1984). There is a lack of information on whether both male and female tree swallows perceive more UV-blue, brighter females as higher quality or higher social status, making these types of experiments worthy of investigation.

5. General Discussion

Sexual selection in now widely accepted as the evolutionary force driving the development of conspicuous plumages in male birds (Andersson 1994), however, the evolution of female plumage ornaments are less understood. Only recently have studies started to examine the signaling potential of female plumage ornaments (Amundsen 2000; Amundsen and Pärn 2006). Contrary to the belief that female plumage ornaments are merely a genetic by-product of selection for these traits in males (Lande 1980), evidence is now accumulating that female plumage ornaments, particularly female plumage colouration, have evolved by male mate choice and/or intrasexual aggressive interactions (Johnsen et al. 1996; Amundsen et al. 1997).

In Chapter 2, I presented new information on the potential adaptive function of DPM in female tree swallows. Only one study, 23 years ago, has experimentally investigated the potential signaling function of DPM in female tree swallows (Stutchbury and Robertson 1987a). Those authors suggested that DPM is adaptive by signaling female-status to males, thus reducing aggression from males during the breeding season, potentially increasing nest-prospecting opportunities for these females (Stutchbury and Robertson 1987a). My results, on the other hand, showed that DPM did not reduce aggression from males; rather DPM appeared to reduce aggression from females when models were presented as intruders into occupied tree swallow territories. Female tree swallows are highly aggressive during the breeding season as they compete for suitable mates and nest sites, and aggressive interactions among females can lead to severe injury or death (Leffelaar and Robertson 1985; CD Coady personal observation). My results suggest that DPM signals subordinate status to competing

females rather than to potential mates, suggesting an intrasexual function of DPM, in accordance with studies that have examined the signaling function of DPM when exhibited by SY males in other species (Greene et al. 2000; Karubian et al. 2008). If I were to repeat my experiments from Chapter 2, where I examined behaviour of resident tree swallows towards the SY and ASY model intruders, I would capture and mark the resident pairs prior to performing the experiments. I would have been able to accurately identify the sex of each resident tree swallow, and not diminish my sample sizes when examining the resident pairs separately by sex. Furthermore, the fact that I was not always able to accurately identify the gender of the residents throughout the entire observation period makes it possible that I missed instances when resident males were aggressive towards either model.

In Chapter 2, I also wanted to address the hypothesis that DPM could be disadvantageous to SY females by advertising their inexperience in nest site defense, resulting in an increased number of intrusions into their territories (Whittingham and Schwabl 2002), potentially reducing their reproductive success if usurped by more experienced ASY females. As I found that the nest stage of neighboring tree swallows influenced the amount of aggressive intrusions into my model territories, the intrusions into my model territories were most likely neighboring tree swallows that were defending their own territories, and were less secure of their own nest site occupancy. Therefore, I was unable to definitively examine if DPM could signal inexperience in nest site defense and be potentially disadvantageous for this species. This question is still worthy of investigation as Whittingham and Schwabl (2002) found that SY female tree swallows experienced more territory intrusions compared to ASY females. Future studies should repeat my experiments and increase the distance of model territories from already established territories or by

creating model territories earlier in the breeding season when most tree swallows have not yet established territories.

In Chapter 3, I investigated the possibility that variation in the expression of DPM could be related to individual quality in SY female tree swallows, a task that to my knowledge has not been examined before in any species. There is a great deal of variation in the amount of DPM exhibited by SY female tree swallows, and as signals of quality are known to have higher degrees of variability than non-signaling morphological traits (Møller and Hoglund 1991; Rowe and Houle 1996), variation in plumage colouration of SY females could be communicating some aspect of their quality. I found that the amount of DPM was not related to any measures of maternal reproductive investment (i.e. clutch initiation date, clutch size, female time spent incubating, female nestling provisioning etc.), suggesting that the amount of DPM exhibited by SY female tree swallows is not a signal of individual quality, at least in terms of female investment in reproduction. I also expected to find that SY females that exhibited more adult-coloured plumage would be in better condition if structurally-coloured plumage is in fact costlier to produce compared to melanin-based plumage colouration (McGraw et al. 2002). I also predicted that SY females with more adult-coloured plumage would have higher reproductive success and would be mated to males with brighter or more UV-blue colouration, as previous work suggests that colouration of ASY female tree swallows may be related to individual quality and may be selected by males (Bitton et al. 2008). SY females that exhibited more brown, subadult-coloured plumage, however, were in better condition, were mated to males with more UV-blue colouration, and raised young that were larger prior to fledging. As I found that resident females were more aggressive towards ASY compared to SY models (Chapter 2), aggressiveness of residents

towards SY females may also depend on variation in the amount of DPM exhibited by SY females. If more adult-coloured SY females are experiencing more aggression from conspecifics, this could result in the reduced body condition and reproductive success of these individuals, as has been found in other species (Grant 1990; Ellis and Good 2006). I did not, however, perform any behavioural observations examining female-female interactions during the breeding season. Although previous studies on different species support my suggestions that more adult-coloured individuals experience increased harassment from conspecifics (Greene et al. 2000; Karubian et al. 2008), future studies need to investigate whether or not this is occurring in tree swallows.

In Chapter 3, I also suggested that variation in the amount of adult-coloured plumage of SY females could be a result of differences in hormones responsible for the acquisition of adult-coloured plumage in this species. Schwarzova et al. (2010) found that SY male black redstarts (*Phoenicurus ochruros*) that moulted into fully adult-coloured plumage had significantly higher plasma testosterone levels during moult compared to individuals that displayed DPM and moulted into subadult-coloured plumage, suggesting a lack of testosterone was responsible for DPM. In tree swallows, studies have suggested that female plumage colouration may be a hormone-dependent trait (McCarty and Secord 2000; Stapleton et al. 2001). If so, variation in hormonal profiles could also be associated with variation in reproductive behavior in SY female tree swallows. SY females with more adult-coloured plumage could have decreased parental investment and decreased choosiness when selecting mates (O'Neal et al. 2008; Veiga and Polo 2008), resulting in the reduced reproductive success of more adult-coloured SY female tree swallows and the negative assortative mating for plumage characteristics in my study. I did not, however, measure

hormone profiles of females in my study, therefore future studies should, at the very least, examine whether there is a correlation between the amount of adult-coloured plumage displayed by females and levels of testosterone. The SY females in my study attained their plumage colouration after their post-juvenile moult at the end of the previous summer (Stutchbury and Rohwer 1990), therefore their current hormone levels do not necessarily reflect their circulating hormone levels when their feathers were grown. Bortolotti et al. (2008) measured the stress hormone, corticosterone, in feathers and found that deposition of this hormone within the feather integument was related to the amount of black colouration in the primary feathers of red-legged partridges (*Alectoris rufa*). Theoretically, one should also be able to quantify the amount of testosterone deposited in feathers as well. Future studies should attempt to examine the relationship between hormones deposited within SY female tree swallow feathers and the amount of DPM expressed for those feathers, potentially identifying the physiological mechanism underlying the variation in the expression of DPM in this species.

Chapter 4 addressed the question of whether variation in plumage colouration within ASY female tree swallows was related to measures of individual quality and could therefore indicate how ASY females choose to balance investment between reproduction and self-maintenance. Although not as apparent as the variation in plumage colouration within SY female tree swallows (Hussell 1983; Chapter 3), I investigated the potential condition-dependence of female plumage colouration in ASY females because only one study has previously examined the potential signaling function of structural plumage colouration in female tree swallows. My findings were somewhat contradictory to those of Bitton et al. (2008) who found that UV-blue colouration varied with age in ASY female tree swallows

and that pairs mated assortatively for plumage brightness, suggesting that structural plumage colouration may be related to female quality and may be a sexually selected trait in this species. Although I also found some suggestion that UV-blue colouration increased with age, I also showed that more UV-blue females had lower reproductive success and that more UV-blue, brighter females had higher amounts of nest parasitism. My findings are also somewhat contradictory to the current literature on ornamental traits being indicators of quality, where more ornamented individuals should be of higher quality and commonly have higher reproductive success (Siefferman and Hill 2005a; Doutrelant et al. 2008) and lower levels of parasitism (Doucet and Montgomerie 2003; Hill et al. 2005). If, however, more UV-blue, brighter plumage is sexually selected in female tree swallows, these females may be experiencing differential costs associated with increased social interactions with potential mates. Furthermore, these females may also be dominant to duller, greener females, as both increased UV-blue colouration and brightness have been found to be associated with higher social status in other species (Reudink et al. 2009; Remy et al. 2010). More UV-blue, brighter females may therefore engage in an increased number of aggressive interactions if their dominance is repeatedly challenged (Goymann and Wingfield 2004). Increased aggressive interactions could potentially result in increased stress levels and/or reduced time spent on self-maintenance and reproductive activities, leading to increased levels of parasitism and reduced reproductive success (Silverin 1986; Hart 1997; Ellis and Good 2006) for these more ornamented females. My findings that increased ornamentation is related to increased amounts of parasitism and reduced reproductive success are rarely reported in the literature. Chapter 4 adds to this small body of literature where more ornamented individuals have been found to suffer from increased parasitism (Burley et al. 1991; Weatherhead et al.

1993) and reduced reproductive success (Griffith et al. 1999; Schroeder et al. 2009). Like mine, these studies are only able to speculate as to the mechanisms driving these unexpected relationships between ornamentation and measures of quality, therefore my results further highlight the need for future studies to experimentally investigate possible reasons for these findings.

Throughout this thesis I have investigated the potential signaling function of plumage characteristics in female tree swallows. Specifically, in Chapters 2 and 3 I investigated the potential signaling function of DPM in female tree swallows and the potential condition-dependence of this trait. In Chapter 2, I demonstrated that DPM has an adaptive intrasexual subordinate signaling function for SY female tree swallows. This finding has never been reported for this species (Stutchbury and Robertson 1987a), however it is a common finding in several species where males exhibit DPM (Lyon and Montgomerie 1986; Rohwer and Butcher 1988). My results are the first to experimentally indicate that the same selective pressures that may drive the expression of DPM in males may also be driving the expression of DPM in females. Further to this finding, my results from Chapter 3 suggest that responses by conspecific females to this signal may vary in relation to the amount of DPM expressed. Rarely has variation in the amount of DPM been measured (Hill 1988; Grant 1990; Greene et al. 2000), or linked to aspects of condition or individual quality, therefore my research greatly contributes to the limited understanding of the mechanisms that drive the evolution of DPM. My results particularly lend further insight into the understanding of the selective forces resulting in the existence of DPM in females, an area of study that is limited due to the few species where this plumage trait occurs in females (Thompson and Leu 1995). Chapter 4 did not examine the subadult plumage of female tree swallows, this chapter also examines

the signaling function of adult-coloured plumage characteristics in females, an area that is in need of more research. In this chapter, I found that higher amounts of ornamentation in ASY females may indicate potential costs of increased parasitism and reduced reproductive success; such results have not often been reported and are worthy of further investigation. Collectively, my results give support to the notion that increased ornamentation can be costly to the condition and reproductive success of female tree swallows. This finding is most likely a result of increased social pressures due to increased female ornamentation. This thesis adds to the growing, yet limited, literature on the signaling function of female plumage characteristics and specifically lends more insight into the potential social consequences of plumage characteristics in female tree swallows.

References

Abbas AK, Lichtman AH (2005) Cellular and molecular immunology. Fifth edition. Elsevier Saunders, Philadelphia

Amundsen T, Forsgren E, Hansen LTT (1997) On the function of female ornaments: male bluethroats prefer colourful females. Proc R Soc Lond B 264:1579–1586

Amundsen T (2000) Female ornaments: genetically correlated or sexually selected? In: Y. Espmark Y, Amundsen T, Rosenqvist G (eds) Animal signals: signaling and signal design in animal communication. Tapir Academic Press, Tapir, pp 133-154

Amundsen T, Pärn H (2006) Female coloration: review of functional and non-functional hypotheses. In: Hill GE, McGraw KJ (eds) Bird coloration. Vol II, Function and evolution. Harvard University Press, Cambridge, pp 280–345

Andersson M (1994) Sexual Selection. Princeton University Press, Princeton

Arcese P (1987) Age, intrusion pressure, and defense against floaters in territorial-male song sparrows. Anim Behav 35:773-784

Ardia DR (2005a) Tree swallows trade off immune function and reproductive effort differently across their range. Ecology 86:2040-2046

Ardia DR (2005b) Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. J Anim Ecol 74:517-524

Ardia DR, Clotfleter ED (2007) Individual quality and age affect responses to an energetic constraint in a cavity-nesting bird. Behav Ecol 18:259-266

Astheimer LB, Buttemer WA, Wingfield JC (1992) Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scand 23:355-365

Balenger SL, Johnson LS, Masters BS (2009) Sexual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebird (*Sialia currucoides*). Behav Ecol and Sociobiol 63:403–411

Beauchamp G (2003) Delayed plumage maturation in birds in relation to social foraging and breeding competition. Evol Ecol Res 5:589-596

Bitton P-P, O'Brien EL, Dawson RD (2007) Male plumage brightness and age predict extra-pair fertilization success in tree swallows (*Tachycineta bicolor*). Anim Behav 74:1777-1784

Bitton P-P, Dawson RD, Ochs CL (2008) Plumage characteristics, reproductive investment, and assortative mating in tree swallows (*Tachycineta bicolor*). Behav Ecol Sociobiol 62:1543-1550

Bokony V, Garamszegi LZ, Hirschenhauser K, Liker A (2008) Testosterone and melanin-based black plumage coloration: a comparative study. Behav Ecol Sociobiol 62:1229–1238

Bortolotti GR, Marchant TA, Blas J, German T (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct Ecol 22:494-500

Burley N (1986) Sexual selection for aesthetic traits in species with biparental care. Am Nat 127:415-445

Burley N, Tidemann SC, Halupka K (1991) Bill colour and parasite levels of zebra finches. In: Loye JE, Zuk M (eds) Bird-parasite interactions. Oxford University Press, New York, pp 359-376

Burtt EH Jr, Chow W, Babbitt GA (1991) Occurrence and demography of mites of tree swallow, house wren, and eastern bluebird nests. In: Loye JE, Zuk M (eds) Bird-parasite interactions. Oxford University Press, New York, pp 104-122

Cohen RR (1984a) Behavioral determinants of nest site tenacity and mate fidelity patterns in tree swallows (*Tachycineta bicolor*). J Colo-Wyo Acad Sci 16:16

Cohen RR (1984b) Criteria for distinguishing breeding male tree swallows from brightly colored females prior to capture. N Am Bird Bander 9:2-3

Cohen RR (1985) Capturing breeding male Tree Swallows with feathers. N Am Bird Bander 10:18-21

Cohen RR (1987) Spring arrival and nest-site acquisition patterns in tree swallows (*Tachycineta bicolor*). Abstr, Am Ornithol Union Mtg San Francisco

Conover MR, Reese JG, Brown AD (2000) Costs and benefits of subadult plumage in mute swans: testing hypotheses for the evolution of delayed plumage maturation. Am Nat 156:193-200

Cooper CB, Mills H (2005) New software for quantifying incubation behavior from time-series recordings. J Field Ornithol 76:352-356

Darwin C (1871) The descent of man, and selection in relation to sex. John Murray, London

Dawson RD, Bortolotti GR (2000) Effects of hematozoan parasites on condition and return rates of American kestrels. Auk 117:373-380

Dawson RD, Lawrie CC, O'Brien EL (2005a) The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. Oecologia 144:499-507

Dawson RD, Hillen KK, Whitworth TL (2005b) Effects of experimental variation in temperature on larval densities of parasitic Protocalliphora (Diptera: Calliphoridae) in nests of tree swallows (Passeriformes: Hirundinidae). Environ Entomol 34:563-568

Dawson RD, O'Brien EL, Mlynowski TJ (2011) The price of insulation: costs and benefits of feather delivery to nests for male tree swallows (*Tachycineta bicolor*). J Avian Biol 42: in press

Deerenberg C, Apanius V, Daan S, Bos N (1997) Reproductive effort decreases antibody responsiveness. Proc R Soc Lond B 264:1021-1029

Doucet SM (2002) Structural plumage coloration, male body size, and body condition in the blue-black grassquit. Condor 104:30-38

Doucet SM, Montgomerie R (2003) Structural plumage colour and parasites in Satin Bowerbirds (*Ptiolnorhynchus violaceus*): Implications for sexual selection. J Avian Biol 34:237-242

Doutrelant C, Gregoire A, Grnac N, Gomez D, Lambrechts MM, Perret P (2008) Female colouration indicates female reproductive capacity in blue tits. Evol Biol 21:226–233

Duckworth RA (2006) Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. Behav Ecol 17:1011-1019

Ellis JC, Good TP (2006) Nest attributes, aggression, and breeding success of gulls in single and mixed species subcolonies. Condor 108:211-219

Enstrom DA (1992) Delayed plumage maturation in the Orchard Oriole (*Icterus spurius*): Tests of winter adaptation hypotheses. Behav Ecol Sociobiol 30:35-42

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139:603–622

Friedl TP, Edler R (2005) Stress-dependent trade-off between immunological condition and reproductive performance in the polygynous red bishop (*Euplectes orix*). Evol Ecol 19:221–239

Goater CP, Holmes JC (1997) Parasite-mediated natural selection. In: DH Clayton, Moore J (eds) Host-parasite evolution. Oxford University Press, New York, pp 9-29

Gold CS, Dahlsten DL (1989) Prevalence, habitat selection, and biology of Protocalliphora (Diptera, Calliphoridae) found in nests of mountain and chestnut-backed chickadees in California. Hilgardia 57:1-19

Goymann W, Wingfield JC (2004) Allostatic load, social status and stress hormones: the costs of social status matter. Anim Behav 67:591-602

Grant BR (1990) The significance of subadult plumage in Darwin's Finches, (*Geospiza fortis*). Behav Ecol 1:161-170

Greene E, Lyon BE, Muehter VR, Ratcliffe L, Oliver SJ, Boag PT (2000) Disruptive sexual selection for plumage coloration in a passerine bird. Nature 407:1000-1003

Griffith SC, Owens IPF, Burke T (1999) Female choice and annual reproductive success favour less-ornamented male house sparrows. Proc R Soc Lond B 266:765-770

Gustafsson L, Nordling D, Andersson MS, Sheldon BC, Qvarnström A (1994) Infectious diseases, reproductive effort and the cost of reproduction in birds. Proc R Soc Lond B 346:323–331

Halkin S, Linville S (1999) Northern cardinal (*Cardinalis cardinalis*). In: A. Poole A, Gill F (eds) The Birds of North America, Vol. 440. Pennsylvania, pp 1-32

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: A role for parasites? Science 218:384-387

Hanssen SA, Hasselquist D, Folstad I, Erikstad KE (2005) Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. Proc R Soc Lond B 272:1039-1046

Hart BL (1997) Behavioural defence. In: Clayton DH Moore J (eds) Host-parasite evolution. Oxford University Press, New York, pp 59-77

Heaney V, Monaghan P (1996) Optimal allocation of effort between reproductive phases: the trade-off between incubation costs and subsequent brood rearing capacity. Proc R Soc Lond B 263:1719-1724

Heeb P, Werner I, Richner H, Kolliker M (1996) Horizontal transmission and reproductive rates of hen fleas in great tit nests. J Anim Ecol 65:474-484

Hegyi G, Rosivall B, Szöllősi E, Hargitai R, Eens M, Török J (2008a) Phenotypic plasticity in a conspicuous female plumage trait: information content and mating patterns. Anim Behav 75:977–989

Hegyi G, Garamszegi LZ, Eens M, Torok J (2008b) Female ornamentation and territorial conflicts in collared flycatchers (*Ficedula albicollis*). Naturwissenshaften 95:993-996

Hill GE (1988) The function of delayed plumage maturation in male Black-headed Grosbeaks. Auk 105:1-10

Hill GE (1989) Late spring arrival and dull plumage: Aggression avoidance by yearling males? Anim Behav 37:665-673

Hill GE, Montgomerie R (1994) Plumage color signals nutritional condition in the House Finch. Proc R Soc Lond B 258:47-52

Hill GE, Doucet SM, Buchholz R (2005) The effect of coccidial infection on iridescent plumage coloration in wild turkeys. Anim Behav 69:387–394

Hill GE (2006a) Female mate choice for ornamental coloration. In: Hill GE, McGraw KJ (eds) Bird coloration.Vol II, Function and evolution. Harvard University Press, Cambridge, pp 137-200

Hill GE (2006b) Environmental regulation of ornamental coloration. In: Hill GE, McGraw KJ (eds) Bird coloration. Vol 1, Mechanisms and measurements. Harvard University Press, Cambridge, pp 507–560

Holst D von (1998) The concept of stress and its relevance for animal behavior. Adv Study Behav 27:1–131

Holberton RL, Hanano R, Able KP (1990) Age-related dominance in male Dark-eyed Juncos: effects of plumage and prior residence. Anim Behav 40:573–579

Houtman AM, Falls JB (1994) Negative assortative mating in the White-throated Sparrow, (*Zonotrichia albicollis*): The role of mate choice and intra-sexual competition. Anim Behav 48:377-383

Hussell DJT (1983) Age and plumage color in female tree swallows. J Field Ornithol 54:312-318

Hyman J, Hughes M, Searcy WA, Nowicki S (2004) Individual variation in the strength of territory defense in male song sparrows: correlates of age, territory tenure, and neighbor aggressiveness. Behaviour 141:15–27

Ilmonen P, Hakkarainen H, Koivunen V, Korpimäki E, Mullie A, Shutler D (1999) Parental effort and blood parasitism in Tengmalm's owl: effects of natural and experimental variation in food abundance. Oikos 86:79-86

Ilmonen P, Taarna T, Hasselquist D (2000) Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proc R Soc Lond B 267:665-670

Järvi T, Bakken M (1984) The function of the variation in the breast stripe of the Great Tit (*Parus major*). Anim Behav 32:590–596

Johnsen TS, Hengeveld JD, Blank JL, Yasukawa K, Nolan V (1996) Epaulet brightness and condition in female red-winged blackbirds. Auk 113:356-362

Johnstone RA, Reynolds JD, Deutsch JC (1996) Mutual mate choice and sex differences in choosiness. Evolution 50:1382-1391

Karubian J, Sillett TS, Webster MS (2008) The effects of delayed plumage maturation on aggression and survival in male red-backed fairy-wrens. Behav Ecol 19:508-516

Kempenaers B, Lanctot RB, Robertson RJ (1998) Certainty of paternity and parental investment in eastern bluebirds and tree swallows. Anim Behav 55:845-860

Kempenaers B, Congdon B, Boag P, Robertson RJ (1999) Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? Behav Ecol 10:304-311

Keyser AJ, Hill GE (1999) Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. Proc R Soc Lond B 266:771-778

Keyser AJ, Hill GE (2000) Structurally based plumage coloration is an honest signal of quality in male Blue Grosbeaks. Behav Ecol 11:202-209

Kodric-Brown A, Brown JH (1984) Truth in advertising: The kinds of traits favored by sexual selection. Am Nat 124:309-323

Korsten P, Dikstra TH, Komdeur J (2007) Is UV signaling involved in male-male territorial conflict in the blue tit (*Cyanistes caeruleus*)? A new experimental approach. Behaviour 144:447-470

Kuby J (1997) Immunology. W.H. Freeman and Co., New York

Kuerzi RG (1941) Life history studies of the tree swallow. Proc Linn Soc NY 52-53:1-52

Lande R (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292-305

Landmann A, Kollinsky C (1995) Territory defence in black redstarts, (*Phoenicurus ochruros*): effects of intruder and owner age? Ethology 101:121-129

Leffelaar D, Robertson RJ (1985) Nest usurpation and female competition for breeding opportunities by tree swallows. Wilson Bull 97:221-224

Leffelaar D, Robertson RJ (1986) Equality of feeding roles and the maintenance of monogamy in tree swallows. Behav Ecol Sociobiol 18:199-206

Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. Auk 104:116-121

Lifjeld JT, Robertson RJ (1992) Female control of extra-pair fertilization in tree swallows. Behav Ecol Sociobiol 31:89-96

Lifjeld JT, Dunn PO, Robertson RJ, Boag PT (1993) Extra-pair paternity in monogamous tree swallows. Anim Behav 45:213-229

Lozano GA, Handford PT (1995) A test of an assumption of delayed plumage maturation hypotheses using female tree swallows. Wilson Bull 107:152-164

Lyon BE, Montgomerie RD (1986) Delayed plumage maturation in passerine birds: reliable signaling by subordinate males? Evolution 40:605-615

Martin II LB, Pless M, Svoboda J, Wikelski M (2004) Immune activity in temperate and tropical house sparrows: a common-garden experiment. Ecology 85:2323-2331

Matson KD, Ricklefs RE, Klasing KC (2005) A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Dev Comp Immun 29:275-286

McCarty JP, Secord AL (2000) Possible effects of PCB contamination on female plumage color and reproductive success in Hudson River tree swallows. Auk 117:987-995

McCarty JP (2002) The number of visits to the nest by parents is an accurate measure of food delivered to nestlings in Tree Swallows. J Field Ornithol 73:9-14

McGlothlin JW, Neudorf DLH, Casto JM, Nolan Jr.V, Ketterson ED (2004) Elevated testosterone reduces choosiness in female dark-eyed juncos (*Junco hyemalis*): evidence for a hormonal constraint on sexual selection? Proc R Soc Lond B 271:1377–1384

McGraw KJ, Hill GE, Stradi R, Parker RS (2001) The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). Physiol Biochem Zool 74:843–852

McGraw KJ, Mackillop EA, Dale J, Hauber M (2002) Different colors reveal different information: How nutritional stress affects the expression of melanin- and structurally based ornamental plumage. J Exp Biol 205:3747-3755

McGraw KJ (2007) Dietary mineral content influences the expression of melanin-based ornamental coloration. Behav Ecol 18:137–142

Merino S, Potti J (1995) Mites and blowflies decrease growth and survival in nestling Pied Flycatchers. Oikos 73:95-103

Møller AP (1991) Parasites, sexual ornaments, and mate choice in the barn swallow. In: Loye JE, Zuk M (eds) Bird-parasite interactions. Oxford University Press, New York, pp 328-343

Møller AP, Hoglund J (1991) Patterns of fluctuating asymmetry in avian feather ornaments: Implications for models of sexual selection. Proc R Soc Lond B 245:1-5

Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Letters 12:75-92

Montgomerie R (2008) CLR: Colour Analysis Programs V. 1.05. Queen's University, Kingston

Morton ES (1989) Female hooded warbler plumage does not become more male-like with age. Wils Bull 101:460-462

Muldal A, Gibbs HL, Robertson RJ (1985) Preferred nest spacing of an obligate cavity-nesting bird, the tree swallow. Condor 87:356-363

Murphy TG, Rosenthal MF, Montgomerie R, Tarvin KA (2009) Female American goldfinches use carotenoid-based bill coloration to signal status. Behav Ecol 20:1348-1355

Nordling D, Andersson M, Zohari S, Gustafsson L (1998) Reproductive effort reduces specific immune response and parasite resistance. Proc R Soc Lond B 265:1291-1298

Norris K (1993) Heritable variation in a plumage indicator of viability in male great tits, (*Parus major*). Nature 362:537-539

Norušis MJ (2000) SPSS Advanced Statistics User's Guide - SPSS Inc, Chicago

O'Brien EL, Dawson RD (2007) Context-dependent genetic benefits of extra-pair mate choice in a socially monogamous passerine. Behav Ecol Sociobiol 61:775-782

O'Brien EL, Dawson RD (2008) Parasite-mediated growth strategies and nutritional constraints in a cavity-nesting bird. J Anim Ecol 77:127–134

O'Neal DM, Reichard DG, Pavilis K, Ketterson ED (2008) Experimentally-elevated testosterone, female parental care and reproductive success in a songbird, the Dark-eyed Junco (*Junco hyemalis*). Horm and Behav 54:571-578

Osorio D, Ham AD (2002) Spectral reflectance and directional properties of structural coloration in bird plumage. J Exp Biol 205:2017-2027

Owen JP, Delany ME, Cardona CJ, Bickford AA, Mullens BA (2009) Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (*Ornithonyssus sylviarum*). Int J Parasit 39:789-799

Owen-Ashley NT, Hasselquist D, Wingfield JC (2004) Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in Song Sparrows. Am Nat 164:490–505

Parker GA (1976) Assessment strategy and the evolution of fighting behaviour. J Theor Biol 47:223–243

Part T (2001) Experimental evidence of environmental effects on age-specific reproductive success: the importance of resource quality. Proc R Soc Lond B 268:2267-271

Penteriani V, Ferrer M, Delgado MM (2011) Floater strategies and dynamics in birds, and their importance in conservation biology: towards an understanding of nonbreeders in avian populations. Anim Conserv 14: in press

Pilz KM, Smith HG, Sandell MI, Schwabl H (2002) Interfemale variation in egg yolk androgen allocation in the European starling. Do high-quality females invest more? Anim Behav 65:841-850

Poiani A, Goldsmith AR, Evans MR (2000) Ectoparasites of house sparrows (*Passer domesticus*): an experimental test of the immunocompetence handicap hypothesis and a new model. Behav Ecol Sociobiol 47:230-242

Price T, Birch GL (1996) Repeated evolution of sexual color dimorphism in passerine birds. Auk 113:842-848

Proctor H, Owens I (2000) Mites and birds: diversity, parasitism and coevolution. Trends in Evol Ecol. 15:358-364

Prum RO (2006) Anatomy, physics and evolution of avian structural colors. In: Hill GE, McGraw KJ (eds) Bird coloration. Vol I, Mechanisms and measurements. Harvard University Press, Cambridge, pp 295–353

Quesada J, Senar JC (2007) The role of melanin- and carotenoid-based plumage coloration in nest defence in the great tit. Ethology 113:640-647

Rasband WS (2009) Image J US National Institutes of Health, Bethesda, Maryland, http://rsb.info.nih.gov/ij/

Reichert SE (1998) Game theory and animal contests. In: Dugatkin LA, Reeve HK (eds) Game theory and animal behaviour. Oxford University Press, New York, pp 64–93

Reid JM, Monaghan P, Nager RG (2002) Incubation and the costs of reproduction. In: Deeming DC (ed) Avian Incubation: Behaviour, environment and evolution. Oxford University Press, Oxford, pp 314–325

Rémy A, Grégoire A, Perret P, Doutrelant C (2010) Mediating male–male interactions: the role of the UV blue crest coloration in blue tits. Behav Ecol Sociobiol 64:1839-1847

Reudink MW, Studds CE, Marra PP, Kyser TK, Ratcliffe LM (2009) Plumage brightness predicts nonbreeding season territory quality in a long-distance migratory songbird, the American redstart (*Setophaga ruticilla*). J Avian Biol 40:34–41

Robertson RJ, Gibbs HL (1982) Superterritoriality in tree swallows: a reexamination. Condor 84:313-316

Robertson RJ, Stutchbury BJ, Cohen RR (1992) Tree swallow. In: Poole A, Gill F (eds) Birds of North America vol. 11, Academy of natural sciences and American ornithologists' union, Philadelphia and Washington DC. pp 28

Rohwer S, Fretwell SD, Niles DM (1980) Delayed maturation in passerine plumages and the deceptive acquisition of resources. Am Nat 115:400-437

Rohwer S (1982) The evolution of reliable and unreliable badges of fighting ability. Am Zool 22:531-546

Rohwer S, Butcher G (1988) Winter versus summer explanations for delayed plumage maturation in temperate passerine birds. Am Nat 131:556-572

Røskaft E, Rohwer S (1987) An experimental study of the function of the red epaulettes and the black body colour of male red-winged blackbirds. Anim Behav 35:1070–1077

Rosvall KA (2008) Sexual selection on aggressiveness in females: evidence from an experimental test with tree swallows. Anim Behav 75:1603-1610

Roulin A, Riols C, Dijkstra C, Ducrest AL (2001) Female plumage spottiness signals parasite resistance in the Barn Owl (*Tyto alba*). Behav Ecol 12:103-110

Rowe L, Houle D (1996) The lek paradox and the capture of genetic variance by condition dependent traits. Proc R Soc Lond B 263:1415–1421

Sandell MI, Smith HG (1997) Female aggression in European starlings during the breeding season. Anim Behav 53:13-23

Schroeder J, Lourenço PM, Hooijmeijer JCEW, Both C, Piersma T (2009) A possible case of contemporary selection leading to a decrease in sexual plumage dimorphism in a grassland-breeding shorebird. Behav Ecol 20:797-807

Schwartz AM, Baird TA, Timanus DK (2007) Influence of age and prior experience on territorial behavior and the costs of defense in male collared lizards. Ethology 113:9-17

Schwarzova L, Fuchs R, Frynta D (2010) Delayed plumage maturation correlates with testosterone levels in Black Redstart (*Phoenicurus ochruros*) males. Acta Ornithol 45:91-97

Senar JC, Copete JL, Martin AJ (1998) Behavioural and morphological correlates of variation in the extent of post-juvenile moult in the siskin (*Carduelis spinus*). Ibis 140:661-669

Senar JC (2006) Color displays as intrasexual signals of aggression and dominance. In: Hill GE, McGraw KJ (eds) Bird coloration. Vol II, Function and evolution. Harvard University Press, Cambridge, pp 87-136

Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317-321

Siefferman L, Hill GE (2003) Structural and melanin coloration indicate parental effort and reproductive success in male Eastern Bluebirds. Behav Ecol 14:855-861

Siefferman L, Hill GE (2005a) Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). Evolution 59:1819-1828

Siefferman L, Hill GE (2005b) UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. Anim Behav 69:67-72

Silverin B (1986) Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period. Gen Comp Endocrinol 64:67-74

Simon A, Thomas D, Blondel J, Perret P, Lambrechts MM (2004) Physiological ecology of Mediterranean blue tits (*Parus caeruleus L.*): effects of ectoparasites (*Protocalliphora spp.*) and food abundance on metabolic capacity of nestlings. Physiol Biochem Zool 77: 492–501

Snoeijs T, Eens M, Van Den Steen E, Pinxten R (2007) Kinetics of primary antibody responses to sheep red blood cells in birds: a literature review and new data from great tits and European starlings. Anim Biol 57:79-95

Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research, second edition. W.H. Freeman and Company, New York

Stapleton M, Dunn PO, McCarty J, Secord A, Whittingham LA (2001) Polychlorinated biphenyl contamination and minisatellite DNA mutation rates of tree swallows. Environ Toxicol Biochem 20:2263-2267

Stearns SC (1992) The evolution of life histories. Oxford University Press, New York

Stutchbury BJ, Robertson RJ (1985) Floating populations of female tree swallows. Auk 102:651-654

Stutchbury BJ, Robertson RJ (1987a) Signaling subordinate and female status: two hypotheses for the significance of subadult plumage in female tree swallows. Auk 104:717-723

Stutchbury BJ, Robertson RJ (1987b) Behavioral tactics of subadult female floaters in the tree swallow. Behav Ecol Sociobiol 20:413-419

Stutchbury BJ, Rohwer S (1990) Molt patterns in the Tree Swallow (*Tachycineta bicolor*). Can J Zool 68:1468-147

Stutchbury BJ (1992) Experimental evidence that bright coloration is not important for territory defense in Purple Martins. Behav Ecol Sociobiol 31:27-33

Studd MV, Robertson RJ (1988) Differential allocation of reproductive effort to territorial establishment and maintenance by male yellow warblers (*Dendroica petechia*). Behav Ecol Sociobiol 23:199-210

Thompson CW, Leu M (1995) Molts and plumages of Orange-breasted Buntings (*Passerina leclancherii*): Implications for theories of delayed plumage maturation. Auk 112:1-19

Torres R, Velando A (2005) Male preference for female foot colour in the socially monogamous Blue-footed Booby, (*Sula nebouxii*). Anim Behav 69:59-65

Veiga JP, Polo V (2008) Fitness consequences of increased testosterone levels in female spotless starlings. Am Nat 172:42–53

Weatherhead PJ, Metz KJ, Bennett GF, Irwin RE (1993) Parasite faunas, testosterone and secondary sexual traits in male red-winged blackbirds. Behav Ecol Sociobiol 33:13-23

Whittingham LA, Dunn PO, Robertson RJ (1994) Do female tree swallows guard their mates by copulating frequently? Anim Behav 47:994-997

Whittingham LA, Schwabl H (2002) Maternal testosterone in tree swallow eggs varies with female aggression - Anim Behav 63:63-67

Williams GC (1966) Natural selection, the cost of reproduction and a refinement of Lack's principle. Am Nat 100:687-690

Williams JB (1996) Energetics of avian incubation. In: Carey C (ed) Avian energetics and nutritional ecology. Chapman and Hall, New York, pp 375-416

Williams JB, Miller RA, Harper JM, Wiersma P (2010) Functional linkages for the pace of life, life-history, and environment in birds. Integ Comp Biol 50: 855-868

Wingfield JC, Ball GF, Dufty AMJ, Hegner RE, Ramenofsky M (1987) Testosterone and aggression in birds. Am Sci 75:602–608

Wingfield JC, Wada M (1989) Changes in plasma levels of testosterone during male-male interactions in the song sparrow, (*Melospiza melodia*): time course and specificity of response. J Comp Physiol A 166:189-194

Winkler DW (1993) Use and importance of feathers as nest lining in tree swallows (*Tachycineta bicolor*). Auk 110:29-36

Zahavi A (1975) Mate selection – a selection for a handicap. J Theor Biol 53:205-214

Zera AJ, Harshman LG (2001) The physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32:95-126